AA

Form Approved Through 2/28/01
OMB No. 0925-0001

Department of Health and Human Services
Public Health Service

Grant Application

Follow instructions carefully.
Do not exceed character length restrictions indicated on sample.

LEAVE BLANK--FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board *(Month, Year)*		Date Received

1. TITLE OF PROJECT
GASTRIC BYPASS IN OBESITY: GHRELIN-RELATED WEIGHT LOSS

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT ☐ NO ☐ YES *(If "Yes," state number and title)*
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — New Investigator ☐ YES

3a. NAME *(last, first, middle)*
Meguid, Michael M.

3b. DEGREE(S)
MD, PhD

3c. SOCIAL SECURITY NO.
Provide on Form Page KK.

3d. POSITION TITLE
Professor of Surgery

3e. MAILING ADDRESS *(Street, city, state, zip code)*
SUNY Upstate Medical University
750 E. Adams Street
Syracuse, NY 13210

3f. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Dept. of Surgery, Neuroscience Program

3g. MAJOR SUBDIVISION
College of Medicine

3h. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: (315) 464-6277
FAX: (315) 464-6237

E-MAIL ADDRESS:
Meguidm@upstate.edu

4. HUMAN SUBJECTS — X No ☐ Yes
4A. If "Yes," Exemption no. or IRB approval date { ☐ Full IRB or ☐ Expedited Review
4b. Assurance of compliance no. M-1303

5. VERTEBRATE ANIMALS — ☐ No X Yes
5a. If "Yes," IACUC approval date 10/1/01
5b. Animal welfare assurance no. A3514-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year--MM/DD/YY)*
From 07/01/02 Through 06/30/07

7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD
7a. Direct Costs ($) 250,000
7b. Total Costs ($) 375,840

8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT
8a. Direct Costs ($) 1,250,000
8b. Total Costs ($) 1,895,840

9. APPLICANT ORGANIZATION
Name: The Research Foundation of SUNY
Address: Office of Sponsored Program Services
P.O. Box 9
Albany, NY 12201-0009

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
Forprofit: → ☐ General ☐ Small Business

11. ORGANIZATIONAL COMPONENT CODE 01

12. ENTITY IDENTIFICATION NUMBER
1-146013200-H4
DUNS NO. (If available)
02-065-7151

Congressional District 21

13. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Robert S. Mason
Title: Contract & Grant Specialist
Address: The Research Foundation of SUNY
P.O. Box 9
Albany, NY 12201-0009
Telephone: (518) 434-7109
FAX: (518) 434-7290
E-mail: Robert.Mason@rfsuny.org

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: David W. Temple
Title: R.F. Endorsing Designee
Address: Research Administration
SUNY Upstate Medical University
750 E. Adams Street
Syracuse, NY 13210
Telephone: (315) 464-5476
FAX: (315) 464-5386
E-mail: TempleD@mail.Upstate.edu

15. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE:
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI / PD IN 3A. *(In ink. "Per signature not acceptable.)*
Michael Meguid (MD PhD)
DATE 9/30/01

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE:
I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 14. *(In ink. "Per" signature not acceptable.)*
David W. Temple
DATE 10/03/01

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

30% of US population are morbidly obese. The most effective treatment to induce sustain weight loss is i) gastric reduction (to create a small gastric pouch) inducing early satiety and minimizing amount of food comfortably ingested, and ii) long Roux-en-Y limb (with a jejuno-jejunostomy) anastomosed to the afferent limb, allowing limited distance for digestion of food by gastric, biliary and pancreatic juice. This minimizes absorption, inducing long-term weight loss. Minimally invasive laparoscopic procedure makes this operation popular. **Hypothesis (Ho):** gastric stapling induces early satiety by reduction in gastric peptide ghrelin which inhibits gastric vagal afferents that relay signals to the hypothalamus that via, amiergic/peptidergic neurotrasmitter interactions, decrease food intake. And, resultant reduced nutrient intake, from malabsorption, directly stimulates activity of hypothalamic "nutroreceptors" involved in efferents influencing food intake, via the sympathetics, vagal, and endocrine outflow, decreasing body weight. Based on compelling and tantalizing preliminary data we test our **Ho** via the **specific aims (SPAims)** by randomly dividing hyperphagic obese Zucker rats into 3groups: Gastric bypass operated study group and two controls, i. Sham operated ad-lib food and the ii. Sham operated pair-fed group. **SpAim #1:** Quantify expression of gastric ghrelin, in relation to changes in food intake, meal size and meal number, after gastric bypass. **SpAim #2:** Investigate role of vagus in early satiety, after gastric bypass, by measuring gastric and hypothalamic ghrelin mRNA and NPY/AGRP mRNA and; dopamine (DA) and serotonin (5HT) concentrations, in relation to food intake, meal size and meal number, with and without vagotomy. **SpAim #3:** Quantify DA and 5HT via in vivo microdialysis, and, mRNA expression of DA-D1, D2 and 5HT-5HT1B and 5HT2C receptors in food intake related hypothalamic nuclei using RT-PCR, in situ hybridization and immunocytochemistry, after gastric bypass. **SpAim #4:** Quantify expression of NPY and agouti-related protein (AGRP), targets of afferent feeding-stimulatory signal of ghrelin, in food intake related hypothalamic nuclei, after gastric bypass. **SpAim #5:** Measure acute or chronic effects of gastric bypass on meal size, meal number, body weight and body composition via carcass analysis. Studies focus on control points concerning effectiveness of gastric stapling in reducing food intake and body weight.

PERFORMANCE SITE(S) *(organization, city, state)*

SUNY Upstate Medical University
Surgical Metabolism and Nutrition Lab (Neuroscience Program)
Department of Surgery
750 E. Adams Street
Syracuse, NY 13210

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below.
Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Michael M. Meguid, MD, PhD	SUNY Upstate Medical University	Principal Investigator
Koseku Ohinata, PhD	SUNY Upstate Medical University	Co-Principal Investigator
Lihua Zhang, MD, PhD	SUNY Upstate Medical University	Senior Research Associate
Yuan Xu, MD	SUNY Upstate Medical University	Research Associate
Irina Makarenko, PhD	SUNY Upstate Medical University	Senior Scientist
Chung Chen, PhD	Syracuse University	Consultant
Akio Inui, MD, PhD	Kobe University	Consultant

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☐ Yes ☐ No

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.
Type density and size must conform to limits and specifications provided in the PHS 398 Instructions.

RESEARCH GRANT

TABLE OF CONTENTS

* SBIR/STTR Phase I applications: Items A-D of the Research Plan are limited to 15 pages.

Appendix *(Five collated sets. No page numbering necessary for Appendix.)*

Check if Appendix is included ☐

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited.

Number of publications and manuscripts accepted for publication *(not to exceed 10)* _____

Other items (list):

BUDGET JUSTIFICATION PAGE
MODULAR RESEARCH GRANT APPLICATION

Initial Budget Period	Second Year of Support	Third Year of Support	Fourth Year of Support	Fifth Year of Support
$250,000	$250,000	$250,000	$250,000	$250,000
Total Direct Costs Requested for Entire Project Period				$1,250,000

Personnel:

Michael M. Meguid, MD, PhD, Principal Investigator (20% effort) is an AMA Joseph B. Goldberg awardee in recognition of his lifetime contribution to the field of surgical nutrition. He has published extensively in reputable basic science journals on the problem of food intake regulation studied in different models, including appetite control in the anorexias of cancer, IBD and sepsis, while, maintaining an effective and productive lab team. Due to a recent back injury requiring an operation, he will not resume his clinical practice but will devote full time to pursue his research. He will thus increase his active participation in and supervision of all aspects of the proposed studies. The salary request for his time does not fully reflect his percent of effort on this proposed project; the difference may be considered as a form of institutional cost sharing. He was certified as completing the intense Molecular Biology Immersion Course at Smith College, MA in 1998, following which he established his own molecular biology laboratory, in support of his research.

Koseku Ohinata, PhD, Co-Principal Investigator (100% effort). Dr. Ohinata trained as a Food Scientist in the Division of Life Sciences, Institute for Food Science, Kyoto University, Japan. Under the tutelage of Prof. Yoshikawa during his graduate years he produced a dozen papers, primarily identifying a variety of peptides which related to the regulation of food intake. He worked in close collaboration with Prof. Inui (see **Letter of Collaborative Support)** on a variety of peptides in mice and applied to this laboratory for his postdoctoral training. His experience includes molecular biological techniques, in situ hybridization methods, and immunohistochemical techniques, in addition to the regular laboratory assay methods.

He will be primarily responsible for assaying and measuring gastric ghrelin, its messenger, as well as the m NPY, AGRP, dopamine, and serotonin, as demonstrated in **SECTION C: PRELIMINARY STUDIES**, and as outlined in great detail in **SECTION D: RESEARCH DESIGN AND METHODS**. The estimated number of samples for the entire experiment is 3,552, and thus this crucial aspect of the proposal is deemed to occupy 100% of his effort, together with the assistance of Dr Makarenko and Dr Zhang.

Lihua Zhang, MD, PhD. Senior Research Associate (80% effort). She is also a trained general abdominal surgeon. Dr. Zhang has worked for four years on the research team. She was extensively training during the past years in molecular biological techniques at Smith College in Northampton, MA, and has received advanced HPLC and microdialysis training. Her responsibilities will include research activities in all five Specific Aims and seven studies, see **SECTION D. RESEARCH DESIGN AND METHODS.** Based on the General Research

Design, **Fig 12** she will assist and supervise all microdialysis studies, harvesting hypothalamic food intake related areas, and in performing all the vagotomies **(see Ext #7).** In addition she will assist in ACREM (rat eater meter) data collection and interpretation. She will supervise Ms. Tada in microdialysis analysis as well. She will also supervise the daily activity of rat eater meter, and when necessary assists Dr Ohinata.

Yuan Xu, MD, Research Associate (100% effort). She joined Dr. Meguid's research team in April 2001. She was specifically hired for her skills as a technically exemplary surgeon with the specific task to develop the "Gastric Bypass Roux-en-Y rat model." As outlined in **SECTION C: PRELIMINARY DATA,** she assembled a "surgical team" dedicated to perfecting and reproducing this model initially in the less expensive Sprague-Dawley rat and then in the Zucker rat, representative of human obesity. The arguments in favor of the Zucker rat as a model for human obesity are outlined in **SECTION B. BACKGROUND AND SIGNIFICANCE.** As documented in Figure 6, her dedication, determination and technical skills plus the assistance of a dependable working surgical team has led to her success in developing and establishing a repeatedly dependable "Gastric Bypass Roux-en-Y rat model," with the desired results of decreased food intake and weight loss as summarized in **SECTION C: PRELIMINARY DATA.** Thus a dependable model which can be used to study **GASTRIC BYPASS IN OBESITY: GHRELIN-RELATED WEIGHT LOSS** exists in this laboratory and has been used successfully to develop data in support of our hypothesis stated in **SECTION A. SPECIFIC AIMS.**

This proposal calls for the study of rats which undergo gastric bypass operation and two control groups: 1) sham-operated ad lib and 2) sham operated pair-fed. A total of 816 rats will be needed. Given a safety margin of 10% for attrition, accidental deaths, anesthetic deaths, etc., Dr. Xu is scheduled to operate on 832 rats during the 5 year tenure of this grant or 166 rats/year (3 rats/wk). Due to her skills, the duration of the operation is 75 min but time is required to prepare the rat prior to the operation and to nurse the rat through anesthesia and the first 7 postoperative days; making this a labor-intensive procedure at 3 rats/wk. As indicated in **SECTION D. RESEARCH DESIGN AND METHODS**, Experiments #2, 3, 4, 6 and 7 will be conducted using the Automated Computerized Rat Eater Meter (ACREM) (see **Methods)** which measures changes in not only food intake but also meal size and meal number. Dr. Xu together with Tomoko Tada, MS, will be responsible for collecting and interpreting the voluminous data produced simultaneously by the 32 ACREM cages, which data will be shared with Dr. Chung Chen, our statistical consultant (see letter of consultative support). She will also be responsible for killing each rat at the end of each study and performing the necropsy.

Irina G. Makarenko, PhD, Senior Scientist (50% effort). She joined Dr. Meguid's research team in January 2001, having been recruited specifically for her skills in neuro-anatomy and immunohistochemistry of the hypothalamus. She is a biologist with specialized skills in neuro-morphology with outstanding experience in this field, as testified by her numerous publications. She will be responsible for carrying out the time consuming immuno-histochemical studies outlined in **Experiment # 3**. This involves confirmation of the specific sites and the amount of peptide and its gene expression, by using immunohistochemistry and in situ hybridization for: ghrelin, NPY, AGRP and dopamine D1, D2, serotonin 5HT1B or 5HT2C receptors in hypothalamus, and for ghrelin in stomach in Zucker rats. Due to the number of rats involved, the estimated number of samples in brain and stomach exceed 218 and it is anticipated that this aspect of these labor intensive studies will take approximate two years, at the stated % effort. She will receive general support in these efforts by Ms. TomokoTada, our technician

Tomoko Tada, MS. Technician (100% effort). Ms. Tada is our only laboratory technician. She has worked in this capacity for the last three years. She is responsible for making up all the solution and ordering chemicals, supplies and for these project 816+ rats. She will assist Dr Xu in preparing the rats for surgery, in monitoring and collecting the ACREM (rat eater meter) data. She will also assist in sample preparation and analysis of glucose, FFA, Triglycerides, Insulin, (a total of 1824 samples) and selected rat Carcass analysis, as outlined in **SECTION D. RESEARCH DESIGN AND METHODS** to accomplish the aims of Specific Aims # 1-5. She will assist Dr. Ohinata, Dr. Zhang and Dr. Makarenko in performing their tasks.

Debra Spadaro, MPA Administrative Research Coordinator (50% effort). Ms. Spadaro will assist and/or coordinate the preparation of research manuscripts for submission, applying knowledge of journal guidelines. Will provide guidance to research staff regarding manuscript guidelines and will assist with communications with authors and the journal's editorial office. She will organize grant project meetings for the Principal Investigator and research staff. She will be responsible for coordinating animal protocol renewals. Will perform grant management activities regarding post-award spending activities and be responsible for the preparation of summary progress reports. Assists all faculty members with conference participation applications; oversees the prepartinon of abstracts and assists with presentation materials including the composition of chars, tables and text slides. She will also perform literature searches for publications.

Consultants:

Chung Chen, PhD. Consultant **(see letter of support)**. (15% effort). Professor Chen is Chairman of the Department of Quantitative Methods at Syracuse University and is a long time collaborator with Dr. Meguid and his group He has assisted in study design, analysis of data, and will continue these tasks on an ongoing basis. In addition, the data generated from the Automated Computer Rat Eater Meter (rat eater meter) necessitates tedious and time-consuming time series analyses. It is estimated that Dr. Chen will fully utilize the equivalent of 15% effort on an ongoing basis throughout the five year tenure of the application in these essential analyses.

Akio Inui, MD, PhD Consultant **(see letter of support)**. (5% effort). Professor Inui is a Clinical Research Scientist in the Division of Diabetes, Digestive and Kidney Diseases, Department of Clinical Molecular Medicine at Kobe University Graduate School of Medicine, Japan. He has been an intellectual collaborator for a number of years and this is the first joint bench research collaboration. As an established researcher in the field of neuropeptides, as these relate to the regulation of food intake in obesity and in anorexia, and as an early worker with ghrelin (see **Biographical Sketch**) he has access and a ready supply to Ghrelin anti-body as well as to other peptide anti bodies needed to elegantly accomplish these studies. In addition the physical presence of his graduate student, Dr Ohinata, in my lab for the next few years, and our continuous intellectual interchange via E-mail, makes this a productive and dynamic collaborative/consultative contribution.

CONSORTIUM/CONTRACTUAL COSTS: NONE

EQUIPMENT:

- **VWR brand Standard Ultra-low Temperature (-86 deg centigrade) Upright Freezer.** Supplied by VWR Scientific 2001 estimated cost $8000.00.

This equipment is essentially needed in the first year of the tenure of the application in keeping with the scope of the grant as outlined in **Fig. 11** (see **SECTION D: RESEARCH DESIGNS AND METHODS**). Our current freezer capacity is reaching a critical limit and would not adequately serve the needs of the samples generated during the tenure of this application.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Michael M. Meguid, M.D., Ph.D.	Principal Investigator Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University College Hospital Medical School University of London, England	MB BS	1968	Medicine
Massachusetts Institute of Technology Cambridge, Massachusetts	PhD	1981	Nutritional Metabolism & Biochemistry

PROFESSIONAL APPOINTMENTS

1970-1972 **Junior Assistant Resident**, Department of Surgery, Harvard Medical School at Peter Pent Brigham Hospital and Children's Hospital Medical Center, Boston, MA

1972-1974 **Research Fellow in Surgical Metabolism**, Surgical Laboratory, Department of General Surgery, Harvard Medical School at Peter Bent Brigham Hospital, Boston MA

1974-1976 **Senior Assistant and Chief Surgical Resident**, University Hospital and Boston City Hospital, Boston University Medical Center, Boston, MA

1976-1979 **Assistant Professor** of Surgery, Boston University Medical Center, Boston, MA

1979-1981 **Research Assistant**, Clinical Research Center, Department of Nutrition and Food Science Massachusetts Institute of Technology, Cambridge, MA

1979-1984 **Director**, Dept. of Clinical Nutrition, City of Hope National Medical Center, Duarte, CA

1982-1984 **Associate Professor**, Dept. of General and Oncologic Surgery, City of Hope National Medical Center, Duarte; University of California, Los Angeles, CA

1984 **Professor**, Dept. of Surgery, University Hospital, and V.A. Medical Center, Syracuse, NY

1994 **Professor**, Dept. of Neuroscience, SUNY Health Science Center Medical School, Syracuse, NY

1997 **Director**, Combined Surgical Research Labs, SUNY Health Science Center, Syracuse, NY

CERTIFICATION:

1980-American Board of Surgery; 1989-Recertified
1982-American Board of Nutrition
1998-Certificate in Molecular Biology, Smith College, Northampton, MA

HONORS

1968 British Medical Student Association, Travel Scholarship to Massachusetts General Hospital, Boston, MA

1970 Postgraduate, Jeremy Cowell Memorial Surgical Scholarship

1981 PhD in Nutritional Biochemistry & Metabolism, Dept. Nut. and Food Sci., MIT Cambridge, MA

1982 Fellow, American college of Surgeons

1982 Member, American Institute of Nutrition

1985 Editor-in-Chief, Nutrition: The International Journal of Applied and Basic Nutritional Science

1986 First Prize in Alpha Omega Research Competition

1989 Regent, American Board of Nutrition, Inc.

1997 Recipient of American Medical Association's Joseph B. Goldberger Award in Clinical Nutrition for 1997

MEMBERSHIPS

American College of Surgeons
FASEB/American Society for Nutritional Sciences
American Society for Clinical Nutrition
Society for the Study of Ingestive Behavior
Society for Surgery of the Alimentary Tract
American Association for the Advancement of Science
North American Assoc. for the Study of Obesity
American Surgical Association
International Behavioral Neuroscience Society
Society for Neuroscience
American Physiological Society

RELEVANT PUBLICATIONS: (selected form 256 peer reviewed papers)

Meguid MM, Kawashima Y, Campos ACL, Gelling P, Hill TW, Chen T-Y, Hitch DC, Mueller J, and Hammond WG. Automated computerized rat eater meter: Description and application. Physiol Behav 1990;48:759-763

Meguid MM, Yang ZJ, and Koseki M. Eating induced rise in LHA-dopamine correlates with meal size in normal and bulbectomized rats. Brain Res Bull 1995;36:487-490

Yang Z-J and **Meguid MM**. LHA-dopaminergic activity in obese and lean Zucker rats. NeuroReport 1995;6:1191

Niijima A and **Meguid MM**. An electrophysiological study on amino acid sensors in the hepato-portal system in the rat. Obesity Res 1995;3(Suppl 5):741S-745S

Yang Z-J, **Meguid MM**, Koseki M, Oler A, Chong C, and Boyd J. Increased food intake and body weight gain after lateral hypothalamic dopaminergic cell implantation. Neuroreport 1996;7:449-453

Meguid MM, Yang Z-J, Laviano A. Meal size and number: relationship to dopamine levels in the ventromedial hypothalamus nucleus. Am J Physiol 1997;272:R1925-R1930

Meguid MM, Fetissov SO, Miyata G, Torelli GF. Feeding pattern in obese Zucker rats after dopaminergic and serotonergic LHA graft. Neuroreport 1999;10:1049-53

Fetissov SO, **Meguid MM**, Shafiroff M, Miyata G, Torelli GF. Dopamine in the VMN of the hypothalamus is important for diurnal distribution of eating in obese male Zucker rats. Nutrition 2000;16:65-66

Varma M, Laviano A, **Meguid MM**, Gleason JR, Yang Z-J and Oler A. Comparison of early feeding pattern dynamics in female and male Fischer rats after reversible VMN block. J Investig Med 2000;48:A417-426

Fettisov SO, **Meguid MM**, Chen C, Miyata G. Synchronized release of dopamine and serotonin in the medial and lateral hypothalamus of rat. Neuroscience 2000;101:657-663

Fetissov S, **Meguid M**, Miyata G, Torelli GF, Shafiroff M. VMN dopaminergic graft and feeding pattern in obese Zucker rats. Int J Obes Relat Metab Disord 2000;24:376-381

Laviano A, Preziosa I, Rossi-Fanelli F, **Meguid MM**. Intracellular energy signals and dietary calcium: a milky way to the physiologic control of hyperphagia and obesity? Nutrition 2001;17:685-685

Sato T, **Meguid MM**, Fetissov SO, Chen C, Zhang L. Hypothalamic dopaminergic receptor expressoins in anorexia of tumor-bearing rats. Am J Physiol Regulatory Integrative Comp Physiol 2001;281:(in press)

MEGUID, M.M.

ONGOING

R01-CA 70239-05 Meguid (PI)	7/15/97-**4/30/02**	20%
NIH/NCI	$225,926	

Serotonin/Dopamine Mediation of Early Cancer Anorexia

This work continues long-term investigation into mechanisms and possible means for amelioration of the anorexia associated with the presence of cancer. The major goal of the project is to elucidate the role of hypothalamic feeding-related monoamine neurotransmitters in the anorexia manifested by tumor-bearing rats.

ONGOING

Meguid (PI)	7//31/01-7/30/03	10%
American Institute of Cancer Research	$46,265	

Cancer Anorexia: Peripheral Manifestation of Abnormal Hypothalamic Neurotransmitter Status Secondary to Cancer

The primary goal of this project is to provide funds to permit the assay of mRNA receptors for serotonin with onset of anorexia in a rat cancer model. No salary support is provided.

COMPLETED

R01DK43796-08 Meguid (PI)	1995-1999	20%
NIH/DK	$278,868	

Rat Model for Surgical Nutrition Research

The goal of this project was to examine and determine the aminergic neurotransmitters involved in the regulation of food intake, meal size and meal number in the obese Zucker rat. These series of studies formed the basis of the Zucker data of the current application.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kosaku Ohinata, PhD	Co Principal Investigator

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tohoku University, Sendai, Japan	BS	1991	Agriculture
Tohoku University, Sendai, Japan	MS	1993	Life Science
Kyoto University, Uji, Japan	PhD	2001	Neuroscience

PROFESSIONAL APPOINTMENTS:

1997-1998 **Special Research Student**, Research Institute for Food Science, Tohoku University, Uji, Japan,
4/2001-6/2001 **Research Fellow**, Japan Society for Promotion of Science, Division of Food Bioscience and Biotechnology, Kyoto University, Uji, Japan
7/2001-present **Research Scientist**, Surgical Metabolism & Nutrition Laboratory, Department of Surgery, Upstate Medical University, Syracuse, NY, USA

HONORS

4/2001-4/2004 Current Award: Japan Society for the Promotion of Science (JSPS)
2001 Ph.D. Title "Studies on Novel Peptides to Decrease Food Intake"

MEMBERSHIPS

Japan Society for the Study of Obesity
Japan Society for Bioscience Biotechnology and Agrochemistry
American Association for the Advancement of Science
American Physiological Society

RELEVANT PUBLICATIONS

Ohinata K, Inui A, Asakawa A, Wada E, Yoshikawa M. Proadrenomedullin N-terminal 20 peptide (PAMP) elevates blood glucose levels via bombesin receptor in mice. FEBS Lett 2000;437:207-211

Ohinata K, Inui A, Asakawa A, Wada K, Wada E, Yoshikawa M. Proadrenomedullin N-terminal 20 peptide (PAMP) inhibits food intake and gastric emptying in mice. Peptides 2001;22:589-595

Ohinata K, Inui A, Askawa A, Yoshikawa M. Novel actions of preadrenomedullin N-terminal 20 peptide (PAMP). Peptides (in press)

Ohinata K, Inui A, Asakawa A, Yoshikawa M. Albutensin A derived from human serum albumin inhibits food intake and gastric emptying. Proceedings of the Twenty First Gut Hormone Conference. (Japanese, in press)

Furukawa Y, **Ohinata K**, Ikai M, Maebashi M, Zhang H, Kimura S. Biotin-stimulated insulin secretion in biotin-deficient rats. J Clin Biochem Nutr 1995;18:35-42

Furukawa Y, Numazawa T, Fukazawa H, Ikai M, **Ohinata K**, Maebashi M, Kim DM, Ito M, Komai M, Kimura S. Biochemical consequences of biotin deficiency in osteogenic disorder shonogi rats. Int J Vitam Nutr Res 1993;63:129-134

The goal of active research and of research conducted during previous three years

The goal of active research is "Screening and development of low molecular peptides to control food intake, analysis of the underlying mechanism and their application". This research is supported by Japan Society for the Promotion of Science (JSPS)

I have studied the subject of "Novel peptides to decrease food intake" over the past three years and have found three novel peptides [albutensin A, complement C3a, and proadrenomedullin N-terminal peptides (PAMP)] that decrease food intake after peripheral and central administration, focusing on their homologies with endogenous peptides. Albutensin A isolated from tryptic digest of serum albumin had homologies with bombesin, C3a and C5a receptors and affinities for their receptors. I demonstrated that the albutensin A-induced anorexia was mediated via C3a receptor using albutensin A-derivatives and bombesin receptor-knockout mice. I also found novel functions of PAMP such as anorexia, hyperglycemia, hypothermia, anxiety and decreases in gastric emptying and metabolism. PAMP had affinity for bombesin receptor and the hyperglycemic effect of PAMP was mediated through bombesin receptor. The PAMP-induced decreases in food intake and gastric emptying were mediated not via bombesin receptor but via a more specific receptor for PAMP using bombesin receptor-knockout mice.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Chung Chen, PhD	Associate Professor of Statistics Chair, Dept. of Quantitative Methods, SU

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
National Taiwan University Taipei, Taiwan	BS	1975	Mathematics
University of Wisconsin-Madison	PhD	1984	Statistics

PROFESSIONAL APPOINTMENTS

1982-1984 **Research Associate**, Graduate School of Business, University of Chicago, Chicago, IL
1984-1988 **Assistant Professor**, Department of Management & Systems, Washington State University, Pullman, WA
1988-1999 **Visiting Assistant Professor**, Department of Quantitative Methods, School of Management, Syracuse University, Syracuse, NY
1989-1992 **Assistant Professor**, Department of Quantitative Methods, School of Management, Syracuse University, Syracuse, NY
1992-present **Associate Professor**, Department of Quantitative Methods, School of Management, Syracuse University, Syracuse, NY
1993-present **Associate Director**, Pacific Area Programs, Kiebach Center for International Business Studies, School of Management, Syracuse University, Syracuse, NY
1994-1998 **Area Coordinator**, Managerial Statistics, Department of Quantitative Methods, School of Management, Syracuse University, Syracuse, NY
1995-1995 **Visiting Associate Professor**, Graduate School of Business, The University of Chicago, Chicago, IL
1998-present **Chairman**, Department of Quantitative Methods, School of Management, Syracuse University, Syracuse, NY

HONORS

1992-present National Scholarship Award, School of Management, Syracuse University, Syracuse, NY
1993-present Associate Editor, *Journal of Business and Economic Statistics*, one of the official journals of the American Statistical Association
1996-present Associate Editor, *Fu Jen Management Review*
1999-present Managing Editor, *Statistica Sinica*
2001-present Associate Editor, *Journal of Data Science*

MEMBERSHIPS

American Statistical Association
International Chinese Statistical Association
Decision Science Institute

APPROVED RESEARCH SUPPORT: Not applicable

RELEVANT PUBLICATIONS:

Chen C, Tiao GC. Random level shift time series models, ARIMA. Approximations and level shift detection. The Journal of Business and Economic Statistics 1990;8:81-96

Chen C, Lee CJ. Vector ARMA test on Gibson Paradox. Review of Economics and Statistics, 1990;LXXII:96-107

Chen C, Liu LM, Tiao GC, Tsay RS. Outlier and intervention analysis in dynamic regression models. Journal of Chinese Statistical Association, 1991:29:1-26 (in Chinese)

Liu LM, **Chen C**. Recent developments of time series analysis in environment impact studies. Journal of Environmental Science and Health, 1991;26:7

Chen C, Liu LM. Joint estimation of model parameters and outlier effects in time series. Journal of American Statistical Association 1993;88:284-297

Chen C, Liu LM. Forecast time series with outliers. Journal of Forecasting, 1993;12:3-35

Chen C, Lee CF, Newbold P, Wu C. On the existence of alternative dynamic daily market models. Advances of Financial Planning & Forecasting. 1994;5:165-180

Chen C, Chang C-H, Koveos P, Zhang YM. Comparative study of economic development: The case of Shanghai and Taiwan. Advances in Pacific Basin Business, Economics and Finance. 1995;1:163-182

Chai J, **Chen C**. Joint efects of measurement errors and grouping on the estimation of moment correlations. Communications in Statistics-Theory and Methods, 1995;24:283-294

Park Y, **Chen C**, Murray TJ. Predicting sun spots using a layer perceptron neural network. IEEE Transactions on Neural Networks, 196;7:501-505

Yang ZJ, Meguid MM, Koseki M, Oler A, Boyd J, **Chen C**. Increase in food intake and body weight gain after bilateral hypothalamic fetal-dopaminergic cell transplantation. NeuroReport 1996;7:449-453

Yang ZJ, Meguid MM, Chai JK, **Chen C**, Oler A. Bilateral hypothalamic dopamine infusion in male Zucker rat suppresses feeding due to reduced meal size. Pharmacology, Biochemistry and Behavior 1997;58:631-635

Fetissov SO,Meguid MM, **Chen C**, Miyata G. Synchronized release of dopamine and serotonin in the medial and lateral hypothalamus of rat. Neuroscience 2000;101:657-663

Sato T, Meguid MM, Fettosov SO, **Chen C**. Amelioration of cancer anorexia resides in the hypothalamus. Surgical Forum 2000;LI:302-304

Sato T, Meguid MM, Quinn RH, Zhang L, **Chen C.** Feeding behavior during sialodacryodenitis viral infection in rats. Physiol Behav 2001;72:721-726

Sato S, Fettisov SO, Meguid MM, Miyata G, **Chen C**. Intra-supraoptic nucleus sulpiride improves anorexia in tumor-bearing rats. NeuroReport 2001;12:2439-2432

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Akio Inui, MD, PhD	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Kobe University School of Medicine Kobe, Japan	MD	1978	Medicine
Kobe University Graduate School Kobe, Japan	PhD	1984	

PROFESSIONAL APPOINTMENTS

1984-1998 **Assistant Professor**, Kobe University, Kobe, Japan
1984-1999 **Chief Physician of the Medical Ward**, Kobe University Hospital, Kobe, Japan
1998-present **Associate Professor**, Department of Clinical Molecular Medicine Kobe University Graduate School of Medicine , Kobe, Japan

HONORS

"Pancreatic polypeptide and insulin secretions"
1997 Awarded Outstanding Researcher in Obesity, Japanese Society for the Study of Obesity
1998 Fellow, International Neuropeptide Society
1999 Editor, Peptides

MEMBERSHIPS

American Endocrine Society
American Diabetic Association
American Gastroenterological Association
American Physiological Society
American Association for the Advancement of Science
International Society for Behavioral Medicine
International Society of Psychoneuroendocrinology
New York Academy of Science
International Association for the Study of Obesity
International Society for Neuroendocrinology
International Neuropeptide Society
International Cytokine Society

RELEVANT PUBLICATIONS

Inui A, Okita M,Najajima M,Momose K, Ueno N, Teranishi A, Miura M, Hirosue Y, Sano K, Sato M, Watanabe M, Sakai T, Watanabe T, Isida K, Silver J, Baba S, Kasuga M. Anxiety-like behavior of transgenic mice with brain expresson of neuropeptide Y. Proc Assn Am Physicians 1998;110:171-182

Asakawa A, **Inui A**, Momose K, Ueno N, Fujino MA, Kasuga M. Motilin increases food intake in mice. Peptides 1998;19:987-990

Inui A. Feeding and body weight regulation by hypothalamic neuropeptides – mediation of the actions of leptin. Trends Neruosci 1999;22:62-67

Inui A. Neuropeptide Y feeding receptors – are multiple subtypes involved? Trends Pharmacol Sci 1999;20:43-46

Inui A. Neuropeptide Y: A key molecule in anorexia and cachexia in wasting disorders? Mol Med Today 1999;5:79-85

Itoh E, Fujimiya M, **Inui A.** Thioperamide, a histamine H3 receptor antagonist, powerfully suppress PYY-induced food intake in rats. Biol Psychiatry 1999;45:475-481

Ueno N, Inui A, Iwamoto M, Kaga T, Asakawa A, Okita M, Fujimiya M, Nakajima Y, Ohmoto Y, Ohnaka M, Nakaya Y, Miyazaki J, Kasuga M. Decreased food intake and body weight in pancreatic polypeptide overexpressing mice. Gastroenterology 1999;117:1427-1432

Asakawa A, **Inui A**, Ueno N, Fujimiya M, Fujino MA, Kasuga M. Mouse pancreatic polypeptide modulates food intake, while not influencing anxiety in mice. Peptides 1999;20:1445-1448

Inui A. Transgenic approach to the study of body weight regulation. Pharmacol Rev 2000;52:35-61

Fujimiya M, Itoh E, Kihara N, Yamamoto I, Fujimura M, **Inui A.** Neuropeptide Y induces fasted pattern of duodenal motility via Y2 receptors in conscious fed rats. Am J Physiol 2000;278:G32-G38

Ohinata K,**Inui A**, Asakawa A, Wada K, Wada E, Yoshikawa M. Proadrenomedullin N-terminal 20 peptide (PAMP) elevates blood glucose levels via bombesin recpetor in mice. FEBS Lett. 2000;473:207-211

Inui A. Transgenic study of energy homeostasis equation: Implications and confounding influences FASEB J 2000;14:2158-2170

Takebayashi Y, Koga H, Togami J, **Inui A**, Kurihara H, Koshiya K, Furuya T, Takanka A, Murase K. Design of the Y1-receptor-selective cyclic peptide based on the C-terminal sequence of neuropeptide Y. J Pept Res 2000;56:409-415

Takebayashi Y, Koga H, Togami J, **Inui A**, Kurihara H, Furuya T, Tanaka A, Murase K. Structure-affinity relationships of C-terminal cyclic analogue of neuropeptide Y for theY1-receptor. Chem Pharm Bull 2000;48:195-1929

Asakawa A, **Inui A**, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 2001;120:337-345

Asakawa A, **Inui A**, Ueno N, Makino S, Fujimiya M, Fujino MA, Kasuga M. Urocortin reduces oxygen consumption in lean and ob/ob mice. Int J Mol Med 2001;7:539-541

Kaga T, **Inui A**, Okita M, Asakawa A, Ueno N,Kasuga M, Fujimiya M, Nishimura N, Dobashi R, Morimoto Y, Liu I-M, heng JT. Modest overexpression of neuropeptide Y in the brain leads to obesity after high sucrose feeding. Diabetes 2001;50:1206-1210

Inui A. Ghrelin: An orexigenic and somatotrophic signal from stomach. Nature Rev Neurosci 2001;2:551-560

Active research during the previous three years

1) Peptides derived from food (governmental support)
Peptides derived from food have effects on biologic functions such as feeding and learning behaviors. I have examined the effects of such peptides administered orally on food intake and energy expenditure in normal animals and obese and diabetic animals for future clinical applications

2) Genetic basis of obesity and diabetes in Japan (governmental support)

To provide the genetic basis in Japanese population, I have examined the association of obesity and simple nucleotide polymorphism on β3 adrenergic receptor, uncoupling protein (UCP), resistin and other factors.

3) Transgenic analysis of obesity and body weight regulation (Japan governmental and non-governmental support)

Transgenic study of energy homeostasis equation is a very effective method to analyze the complicate system of body weight regulation. I have developed several transgenic models such as NPY- or PP-overexpressing mice and brain-specific stat 3 knockout mice, and examined their roles in energy homeostasis

4) Identification of neuropeptide Y feeding receptors (Non-governmental support)

It is still to be determined which and how many neuropeptide Y receptors are responsible for potent feeding-stimulatory effects of neuropeptide Y. I have examined the importance of neuropeptide Y, Y1 and Y5 receptors, as well as the potential involvement of other receptor subtypes such as peptide YY-preferring receptors.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Irina Makarenko, PhD	Senior Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Russian Academy of Sciences, Moscow	PhD	1985	Embriology and Histology

PROFESSIONAL APPOINTMENTS

1974-1980 **Junior Investigator**, I.P. Pavlov Institute of Physiology, USSR Academy of Sciences, Leningrad
1980-1991 **Junior Research Investigator**, All Union Scientific Center of Mental Health USSR, Academy of Medical Sciences, Moscow
1991-1999 **Scientific Research Investigator**, Institute of Development Biology, Russian Academy of Sciences, Moscow
2000-present **Senior Scientist**, Institute of Developmental Biology, Russian Academy of Sciences, Moscow

RELEVANT PUBLICATIONS:

Sergeeva IG Connections between field 17 of the optic cortex/cerebrum/and the mammillary nuclei in cats. Neuroscience and Behavioral Physiology 1980;10:17-21

Otellin VA, Rybakov VL, **Sergeeva IG**, Baikovskaya MN. Development of anterograde degeneration in some projection systems of the brain. Folia Morphologica 1980;28:330-332

Otellin VA, **Sergeeva IG.** Autoradiographic study of the connections between field 17 of the visual cortex and the hypothalamus in the cat. Neuroscience and Behavioral Physiology 1982;12:428-434

Makarenko IG, Kushner SG, Tennnov AV, Dmitriev AD. Distribution of alpha-, beta- and gamma-endorphins in the forebrain and diencephalons of the rat immunohistochemical investigation. Arkhiv Anat Gistol Embriol 1985;89:10-18 (in Russian).

Burbaeva GS, Aksenova MV, **Makarenko IG**, Kalinenko OO. The decrease of creatine kinase-BB content in the brain of mental patients/complex immunochemical and immunocytochemical investigation. Zurn Nevropatol Psikhiat 1990;90:49-52 (in Russian)

Burbaeva G, Aksenova M, **Makarenko IG**. The new marker of neurofibrillary tangles in Alzheimer's disease brain. Biological Psychiatry 1990;29(11S):473S

Makarenko IG. Immunochemical visualization of creatine kinase-BB in the human neocortex. Zhurn Nevropatol Psikhiat 1991;91:38-42 (in Russian)

Popov AP, **Makarenko IG**, Akmaev IG. Immunohistochemical investigation of glial fibrillary acid protein localization in the rat mediobasal hypothalamus in postnasal ontogenesis. Morphologiya 1992;103:59-68

Burbaeva GS, Aksenova MV, **Makarenko IG**. The decrease of immunoreactivity of creatine kinase BB in Alzheimer's disease brains. Dementia 1992;3:91-93

Natochin YV, Meschcherskii IG, Concharevskaya OA, **Makarenko IG,** Shakmatova EI, Ugrumov MV, Feoktistova NY, Alonzo G. Comparative studies on the osmoregulatory system in the hampsters Phodopus roborovskii and Phodopus sungorus. Zhurn Evol Biokhim Fiziol 1994;30:344-357 (in Russian)

Makarenko IG, Aksenova MV, Burbaeva GS. Distribution of creatinine kinase BB in the hippocampus of Alzheimer's patients. Dementia 1992;3:91-93

Shabalov VA, Ugrumov MV, Fedorova NV, Shtok VN, Popov AP, Melikjan AG, Fetisov SO, **Makarenko IG**, Lutsenco CV, Kusin IP, Archipova NA, Safronov VA. Russian experience on neurotransplantation in patients with Parkinson's disease (PD). In: 5th International Symposium on Neural Transplantation, June 25-29, France, 1994.

Makarenko IG, Trembleau A, Derrer P, Calas A, Ugrumov MV. Development of the hypothalamic vasopressinergic system in rats during ontogenesis. Abstracts of International Conference "Mechanisms of development: ontogenetic and phylogenetic aspects" Ontogenez 1994;25:83

Ivanova IP, Radomicheva TV, **Makarenko IG**, Ugrumov MV. Proliferation of the endothelium of primary portal plexus of the hypothalamo-pituitary circulation during ontogenesis in rats. Bull Exp Biol Med 1995;N5:462-464

Makarenko I, Ugrumov M, Derer P, Calas A. Dil tracing study of axonal projections to the median eminence and posterior lobe in prenatal rats. Int J Devel Neuroscience 1996;14(Suppl 1):89

Makarenko I, Ugrumov M, Calas A. Development of connections of magnocellular nuclei of hypothalamus and posterior lobe of pituitary in prenatal ontogenesis of rats. Ontogenez 1999;30:296-301

Makarenko IG, Ugrumov MV, Calas A. Axonal projections from the hypothalamus to the median eminence and posterior lobe in rats during ontogenesis: Dil tracing study. Fifth IBRO Congress of Neuroscinece, Jerusalem, Israel July 11-15, 1999, Abstracts, p 74

Qi Y, Zhang L, **Makarenko IG**, Sato T, Laviano A, Tada T, Chen C, Meguid MM. Hypothalamic link of aminergic to peptidergic neuromodulators in cancer anorexia. American College Surgeons Clinical Congress, New Orleans, October 2001, Surgical Forum 52:X-X, 2001.

Pronina TS, Ugrumov MV, Calas A, Tramu G, **Makarenko IG**. Serotonin influence on the development of the GN-RH system in Wistar rat fetuses. Zhurn Evol Biokhim Fiziol 2001;37:426-430 (in Russian)

Meguid MM, **Makarenko IG**, Ugrumov MV. Neuropeptide Y (NPY) producing systems in the forebrain of rats with cancer anorexia. 31st Annual Meeting Society for Neuroscience, San Diego, California, November 10-15, 2001 (in press)

Makarenko IG, Ugrumov MV, Calas A. Axonal projections from the hypothalamus to the median eminence in rats during ontogenesis: DiI tracing study. J Anat Embryol, 2001(in press)

Ershov PV, Ugrumov MV, Calas A, **Makarenko IG**, Krieger M, Thibault J. Neurons expressing enzymes of dopamine synthesis in the mediobasal hypothalamus of rats: topographic relations and axonal projections to the median eminence in ontogenesis. J Comp Neurol 2001 (in press.)

The goals of active research and research conducted during the previous three years.

Dr. Makarenko is currently carrying out immunocytochemical studies of the distribution and/or colocalization of NPY, dopamine receptors (D1, D2), serotonin receptors (5HT 1A and 1B) in the hypothalamus of normal rats in comparison with **obese** rats in the laboratory of Dr. Michael Meguid. She is currently in his laboratory as a Visiting Scholar appointed under the Research Faculty Development Program of the Office of International Affairs, NIH/NCI.

COMPLETED

Makarenko IG (PI)
"Development of the Hypothalamic Projections to the Median Eminence and Pituitary During Perinatal Ontogenesis in Rats:
Russian Academy of Sciences and Russian Foundation of Basic Research
Period: 1/01/97-12/31/2000

This project examined the ontogenetic schedule of the arrival of the axons of the hypothalamic neurons in the median eminence and pituitary in rats by using the fluorescent lipophilic carbocyanine dye (Dil) as a retrograde tracer. It was shown that this process begins very early in ontogenesis providing the pathway for the neurohormone transfer to the hypophysial portal circulation and general circulation in the posterior pituitary lobe.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Lihua Zhang, MD, PhD	Senior Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Nanjing Medical University	MD	1984	Medicine
Shanghai Secondary Military University Shanghai, China	PhD	1992	Surgery

PROFESSIONAL APPOINTMENTS

1979-1984 **Medical Student**, Nanjing Medical University, Nanjing,China
1984-1987 **Resident,** Intensive Care Unit, Jiangsu Province People's Hospital, China
1987-1992 **PhD Student**, Shanghai Second Military University, Shanghai, China
1992-1994 **Surgeon,** Department of General Surgery, Nanjing Jinling Hospital, Nanjing, China
1994-1997 **Research Fellow**, Department of Clinical Chemistry, Royal Liverpool University Hospital, England
1997-1999 **Associate Professor**, Department of General Surgery, Nanjing Jinling Hospital, Nanjing, China
1999-2000 **Research Fellow**, Neuroscience Program, Surgical Metabolism and Nutrition Laboratory, Department of Surgery, University Hospital, SUNY Upstate Medical University, Syracuse, NY, USA
2000-2001 **Lab Manager/Researcher**, Neuroscience Program, Surgical Metabolism and Nutrition Laboratory, Department of Surgery, University Hospital, SUNY Upstate Medical University, Syracuse, NY, USA

DOCTORAL THESIS

Nutritional support in surgical patients

CERTIFICATION

1998 Molecular Biology and PCR Summer Workshops, New England Biolabs, Northampton, MA 01063

HONORS AND STIPENDS

1995 The Third Award of Science and Technology awarded by the National Scientific and Technological Committee of P. R. China (Certificate 15-3-029-03)
1995 The Fourth Award of Science and Technology awarded by the Rear-Service Department of Nanjing Command of P.L.A. (Certificate 95-4-066-05)
1995 The Second Award of Scientific and Technology awarded by the National Education Committee of P.R. China (Certificate 95-660)
1993 The Second Award of Science and Technology awarded by the General Rear-Service Department of P.L.A. (Certificate 93-2-41-3)
1992 Excellent Paper Award awarded by the Surgical Association of Jiangsu Province, P. R. China
1990 The Second Award of Excellence awarded by the Fifth Conference of National Middle and Youth Doctors of Chinese Medical Association

MEMBERSHIPS

American Physiology Society — Neuroscience
Society of Study of Ingestive Behavior

RELEVANT PUBLICATIONS

Yin L, **Zhang LH**, et al. Sandostatin promotes spontaneous closure of gut fistula. Clin Gen Surgery 1995;10:108-110

Tang WJ, **Zhang LH**. Value of nutritional support in the treatment of severe post-operative inflammatory obstruction of intestine. Parenteral and Enteral Nutrition 1995;2:179-181

Zhang LH, Roberts NB, and Shenkin A. Effect of hyperlipidaemia on plasma vitamin K concentrations. Clin Nutr 1996;15(suppl 1):41-42 (abstract)

Zhang LH, Li JS. The development of lipid formula in nutrition support. Parenteral and Enteral Nutrition 1998;5:33-36

Zhang LH. The development of nutrition reagents in application and research field. Chinese Journal of Practical Surgery 1998;18:762-754

Zhang LH. The development of amino acid solutions in nutrition support. Parenteral and Enteral Nutrition 1998;5:165-167

Zhang LH. The sixth conference summary of Chinese clinical nutrition support. Parenteral and Enteral Nutrition 1999;6:49-51

Zhang LH, Zhang XQ and Li JS. The effect of enteral nutrition IMPACT on immune function of rats with trauma. Parenteral and Enteral Nutrition 1999;6:155-157

Zhang L, Varma M, Meguid MM. Changes in hypothalamic monoamines with nicotine in menopausal females: Implications in food intake and body weight regulation. Surg Forum 2001;LI:419-421

Meguid MM, Fetissov SO, Varma M, Sato T, **Zhang L**, Laviano A and Rossi-Fannelli F. Hypothalamic dopamine and serotonin in the regulation of food intake. Nutrition 2000;16:843-857

Varma M, Sato T, **Zhang L**, Meguid MM. Space flight related anorexia. Lancet 2000;356:681, 2000

Sato T, Meguid MM, Quinn RH, **Zhang L**, Chen C. Feeding behavior during sialodacryoadenitis viral infections in rats. Physiol Behav 2001;72:721-6

Zhang L, Meguid MM, Miyata G, Varma M, Fetissov SO. Role of hypothalamic monoamines in nicotine-induced anorexia in menopausal rats. Surgery 2001;130:133-42

The goal of active research and of research conducted during the past three years:

The main goal of the present and past two years of research has been to elucidate central mechanisms responsible for the development of food intake disorders using male and female rats using nicotine as a model. In vivo microdialysis and molecular biology methods to measure dopamine and serotonin receptor expression were used to obtain insights into the anorectic mechanism.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format (on preceding page) for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Yuan Xu, MD	Research Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The Capital Medical University Beijing, P.R. China	MD	1983	Surgery

PROFESSIONAL EXPERIENCE

1983-1987 **Resident Surgeon**, Department of Surgery, Tong Ren Hospital, Beijing, PR China
1988-1993 **Attending Surgeon**, Department of General Surgery, Tong Ren Hospital, Beijing, PR China
1994-1998 **Associate Professor**, Surgical ICU, Tong Ren Hospital, Beijing, PR China
1999-6/2001 Professor, Surgical ICU, Tong Ren Hospital, Beijing, PR China
7/2001-present **Research Fellow**, Surgical Nutrition and Metabolism Laboratory, University Hospital, SUNY Upstate Medical University, Syracuse, NY, USA

MEMBERSHIP

Chinese Medical Association
Chinese Pathophysiology and Critical Care Medical Association
Chinese Society of Parenteral and Enteral Nutrition
Beijing Society of Parenteral and Enteral Nutition

HONORS

2001 Scientific Talent Training Award: Tong Ren Hospital, Capital Medical University, PR China

RELEVANT PUBLICATIONS

Xu Y, He W, Ge Q, et al. Enteral nutrition-related gastrointestinal complications in critical ill patients in the SICU. Parenteral and Enteral Nutrition 2001;8:151

Zhou H, **Xu Y**, Ge Q, et al. Infective complications of central vein catheterization. Parenteral and Enteral Nutrition 2001;7:38

Ge Q, **Xu Y**, Zhou H, et al. Multiple organ failure induced by ARDS. Chinese J Practical Medicine 2000;2:36

Xu Y. Nutrition support in the patients with obstructive jaundice. Chinese J Practical Surgery 1998;18:723

Xu Y, Lin D. Application of nutrition support in surgical critically ill patients. Chinese J Practical Surgery 1996;16:711

Yu Z, **Xu Y**, Hou J, et al. Immune function in patients with malignant obstructive jaundice and effect of PN on them. Parenteral and Enteral Nutrition 1996;3: 193

Hou J, **Xu Y**, LI T, et al. Postoperative complications and perioperative management of the patients with obstructive jaundice. Chinese J Practical Surgery 1996;16:98

Li T, Xu Y, Hou J, et al. The effect of analgesia with trauma in post-operation. Chinese J Pain Medicine 1996;2:89

Xu Y, Yu Z, Chen F, et al. The metabolic effect of TPN support for the patients with malignant obstructive jaundice in post-operation. Parenteral and Enteral Nutrition 1994;1:45

Xu Y, Zhang L, Li J. Study of clinical application of Chinese-produced 20% Intralipid. Clin J General Surg 1993;8:39

Xu Y and Li J. Nutrition support in patients with obstructive jaundice. Clin J General Surg 1992;7:308

Xu Y and Gong J. The diagnosis and treatment for fistula and sinus of breast. Clin J General Surg 1987;2:229

Xu Y and Gong J. Perioperative management of the diabetic patient. Clin J General Surg 1983;8:251

Xu Y. Fasciculi of Surgery, Multi-choice praxis on clinical medicne. In Gong JZ et al, eds. General. Peking Union Medical College Publishing House, PR China 1999; pp 1-15

Xu Y 580 Frequently asked questions for attending surgeons. In: Trauma. (Zhang S. Y. et al), (1998) 1st ed., (1999) 2nd ed., co-published by Beijing Medical University and Peking Union Medical College Publishing Houses, pp 1-14,

Xu Y. 600 Q & A for attending surgeons on intensive-care cases. In: Metabolism and Nutrition Support, (Liu D. W., et al, eds, co-published by Beijing Medical University and Peking Union Medical College Publishing Houses. 1998, pp 445-516

Xu Y. Practical operation on outpatient department. In: Xu H. J. et al, eds. Putting and Nursing of Central Venous Catheter, 1st ed.People's Hygiene Publishing House,1999, pp 44-56

Xu Y as the translator *Abernethy's Surgical Secrets – Questions you will be asked...* (Alden H., MD, Ernest E. Moore, MD), In: General, (Xu H.J. et al), 1st ed., , co-published by Hong Kong Kewen Publishing Co. Ltd. and Haiyi Publishing House. 1999, pp 1-89

Xu Y Critical care medicine. In: Liu D. W., et al, eds. Metabolic Changes and Nutrition Management of Critically Ill Patients. 1st ed., co-published by Peking Union Medical College Publishing Houses, 2000

Active research during the past three years

Investigated gut permeability in response to to different parenteral and enteral nutrients in critical care patients. Investigating the effects of gastric bypass operation on ghrelin and food intake and body weight loss.

Experimental techniques using rat model with abdominal sepsis and receiving intravenous nutrition; examining intestinal ischemia and reperfusion injury using rat model under different ischemic circumstances. Computer-based experiment data classifying, analyzing and evaluating techniques

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: Please refer to "A" attached.

Clinical: N/A

Animal: Please refer to "B" attached

Computer:

Office: The laboratory is fully equipped with computer techniques including 6 Pentium computers, two laser printers, one color jet printer, high-resolution scanner. SUNY Upstate Medical University has a SEL Concept 32 mainframe computer. This is a dual density 800/1600 BPI system with 500 megabyte disk storage.

The laboratory staff have seating facilities in two rooms of the Neuroscience Program Surgical Metabolism and Nutrition Laboratory.

Other:

An on-line Medlar search computer facility is available in the Department of Surgery.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

A thermocycler, sonic dismembrator, refrigeratingcentrifuge, vibratome, hybridizationoven, spectrophotometer, UV transilluminator, and Nanopure water purification system are available in the Surgical Metabolism and Nutrition Laboratory.

A. **LABORATORY**: The Surgical Metabolism & Nutrition Laboratory (SMNL) consists of three rooms with a total of
1530 sq. ft. of space. The SMNL is situated in the Research Wing of the Department of Surgery at University Hospital of the SUNY Upstate Medical University at Syracuse. It houses both equipment and laboratory and research personnel. The biochemical room has all the standard lab equipment required for preparation and analysis of samples. It houses the microdialysis equipment and it's HPLC, a dual pump HPLC with electrochemical detector, desktop-, refrigerated-, and high speed centrifuges, refrigerators, and freezers. Other equipment include a Ethicon Auto Analyzer, a Kjeldahl digestor and minor equipment in the form of pH meters, water baths, magnetic stirrers, etc. k We also have a water purified, isolation hoods, and a cold room. In the other lab space, we have a scanning densitometer, UV spectrophotometer, a fluorescence spectrophotometer, DU-8 gas chromatograph, analytic balances: gamma counter, low speed and high speed centrifuges. Perfusion pump Masterflex, Fiber-Lite high intensity illuminator, Vibratom and criocut, Fisher Steriomaster Zoom Microscope, Transmission Olympus AH-2 Microscope with attached computer, Fluorescent Olympus Microscope with attached computer, Fluorescent Olympus Microscope with double filter sets (for fluoresceine and rhodamine) for neuromorphological studies. Next to the SMNL there is a fully functional animal operating room which is equipped with operating tables, operating lights, etc. We routinely perform animal operating procedures of different magnitudes under strict aseptic and antiseptic conditions. The other investigative surgeons in the department share this room. In the biochemical lab is a scrub sink, a gas sterilizer, glass washing facilities, liquid nitrogen tanks, and Mettler balances.

B. DESCRIPTION OF ANIMAL FACILITIES. The animal facility is divided into two locations joined by an inside passageway, approximately 14,380 sq. ft. on the fourth floor of Weiskotten Hall and 11,250 sq. ft. on the seventh floor of the south wing of University Hospital. These facilities include wall, partition widths, and hallways. Functions include care and management and procurement of teaching and research animals, teach programs for animal caretakers, and information programs for public school students, assistance to public zoos, and cooperative programs with other units of the State University of New York. 1) Feeding and cleaning of animals, cages, equipment, and rooms; 2) Procurement of animals, feed, cages and ancillary equipment; 3) Assistance to the investigator in the choice of animals and equipment for special needs; 4) Diagnostic services; 5) Staffing and equipping animal surgery and provision for post-op care and 6) conditioning and treatment of animals used for teaching and research. Facilities exist for cage washing and sterilization. Enough space is available that mixing of species is not permitted. All rooms have environment control including air conditioning, air ventilation, and no recirculation of exhaust air. A well-trained staff provides 7 days/week care. We are committed to standards as described in "Guide for the Care and Use of Laboratory Animals, " of the Institute of Laboratory Animal Resources National Research Council. Our Public Health Service assurance number is A3514-01. We are licensed by the U.S. Department of agriculture under license number 21-136, as an approved research facility in compliance with Public Law 89-544 as amended. We are also licensed and registered by the N.Y.S. Department of Health under Section 504 of the Public Health Law. Regular and unannounced inspections are made by several agencies and appropriate annual reports are regularly submitted. We are also committed to the U.S. Interagency Research Animal Committee, Principles for the Utilization and Care of Vertebrate Animals Used in Testing, research and Training. A Committee for the Humane Use of Animals was established per regulations published in Vol. 14, NO. 8, 6/25/85, Special Editions for Grants and Contracts. One member of the committee is a veterinarian who makes rounds on all animals at least three times per week and reviews all protocols before projects are initiated. In addition, other veterinary consultants have been employed. Rounds are also made daily. Diagnostic facilities are provided by Cornell Veterinary College, private laboratories, and various in-house facilities. Facilities available for housing animals are well designed, managed, and maintained.

GASTRIC BYPASS IN OBESITY: GHRELIN-RELATED WEIGHT LOSS

SECTION A. SPECIFIC AIMS

Based on body mass index criteria of 25.0 to 29.9, approximately 25-33% of the US population is overweight, while another 30% are obese (1). And, as expanded on in the **Section B. Background,** obesity continues to be a major medical as well as public health problem, with no effective treatment in sight. As a last resort, the treatment of morbid obesity has been relegated to the surgeon (2,3). The progression of the different types of operations performed for morbid obesity since 1966 is also outlined in the **Section B. Background.** The most successful operation to induce sustained weight loss in the morbid obese, with the fewest metabolic complications, is a procedure that encompasses two basic metabolic/nutritional principles. i) gastric reduction (to create a small gastric pouch with a gastro-jejunostomy): to induce early satiety and to minimize the amount of food that can be comfortably ingested, and ii) a Roux-en-Y limb (with a jejuno-jejunostomy) anastomosed to the afferent limb, thereby allowing only a limited distance for co-mingling of food with gastric, biliary and pancreatic juice, as shown schematically in **Figure 1**.

This minimizes the opportunity for digestion and absorption, inducing long-term weight loss. The ability to perform this operation via minimally invasive laparoscopic procedure has made this operation very popular. But although gastric bypass procedures in general are a successful operation, concern exist re- the frequent lack of inadequate patient follow up (3). Despite this seemingly physiological approach to long term weight loss, comprising of gastric reduction and a component of malabsorption, this surgical treatment for morbid obesity carries a 10 to 20% long-term complication rate and a significant mortality risk.

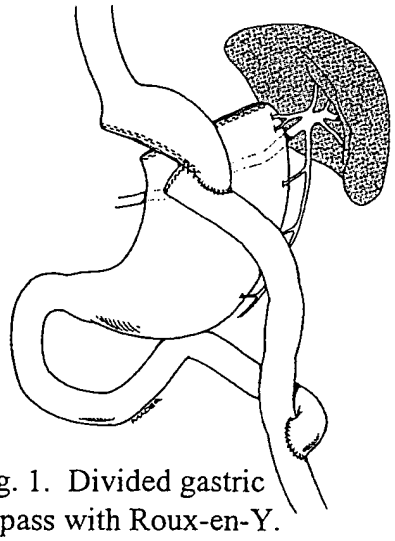


Fig. 1. Divided gastric bypass with Roux-en-Y.

How the operation works in inducing the metabolic/physiologic regulation in food intake, given that food intake is ultimately regulated by the interaction of the classical neurotransmitters (dopamine and serotonin) and the numerous neuropeptides (eg. neuropeptide Y, aguti-related peptide etc) in the hypothalamus, is unknown.

This is the aim and scope of our proposal, based on our **hypothesis** outlined below, which is supported by extensive data presented in **Section B. Background,** and by compelling and tantalizing data generated in this laboratory and presented in **Section C. Preliminary Studies.**

HYPOTHESIS: We hypothesize that gastric stapling, to create a small gastric pouch, induces early satiety by a reduction in the gastric peptide ghrelin (4-11) which inhibits gastric vagal afferents (8) that relay signals to the hypothalamus, that via the interaction of amiergic and peptidergic neurotrasmitters (6,8,12,13) decrease food intake (4-12). And, the resultant reduced nutrient intake, consequent to the induced malabsorption, may also directly affect the activity of "glucoreceptive" and other "nutro- receptive" cells in the hypothalamus involved in the control of the efferents which influences food intake, via the sympathetics, vagal, and endocrine outflow, decreasing body weight.

Procedures for testing our hypothesis are the **specific aims** of this proposal, enumerated below and conceptualized in **Figure 2.** These will be carried out in part in Sprague Dawley (SD) rats to strengthen and enhance our normal data base, particularly because there are limited scientific data on this novel and recently discovered peptide/neuropeptide "Ghrelin". And, then in the obese hyperphagic Zucker rat model. The rational for selecting the obese Zucker rat is outlined in the **Section B. Background.**

Each study requires that the (SD) and Zucker rats be randomly divided into three different experimental groups, identified as:

- Gastric bypass operated study group (**Gastric bypass**) and two controls, the
- i. Sham operated ad-lib food (**Sham ad lib**) and the
- ii. Sham operated pair-fed group (**Sham Pair-Fed**).
-

SPECIFIC AIMS. In the study and control groups defined above and as shown in **Figure 2**:

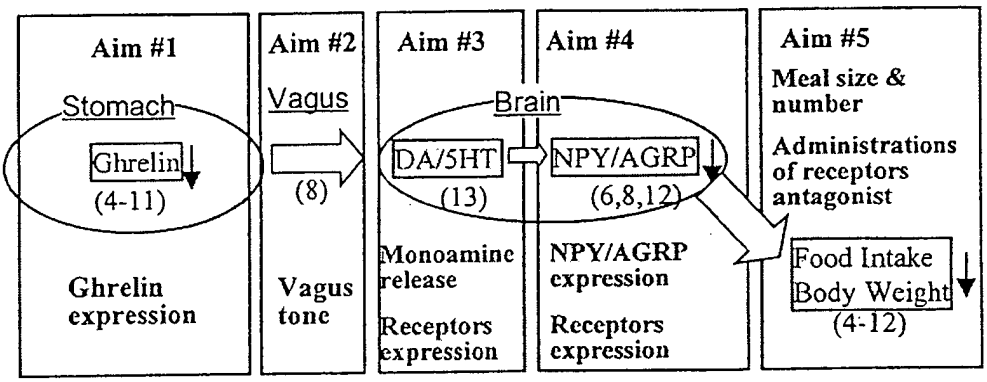


Fig. 2. Hypothesis on the decrease in body weight after gastric bypass operation. (Numbers in parenthesis are references)

Specific Aim #1: To quantify the expression of gastric ghrelin, as it relates to changes in food intake, meal size and meal number, after gastric bypass operation.

Specific Aim #2: To investigate the role of the vagus in early satiety after gastric bypass operation. By measuring gastric ghrelin mRNA, and hypothalamic ghrelin mRNA and NPY/AGRP mRNA as well as dopamine (DA) and serotonin (5HT) concentration, as these relate to changes in food intake, meal size and meal number, with and without vagotomy.

Specific Aim #3: To quantify changes in dopamine (DA) and serotonin (5HT) concentrations via in vivo microdialysis. And, the mRNA expression of dopaminergic D1, D2 and serotonergic 5HT1B and 5HT2C receptors in food intake related hypothalamic nuclei using RT-PCR and in situ hybridization and immunocytochemistry, after gastric bypass operation.

Specific Aim #4: To quantify expression of neuropeptide Y (NPY) and agouti-related protein (AGRP), targets of afferent feeding-stimulatory signal of ghrelin, in food intake related hypothalamic nuclei, after gastric bypass operation.

Specific Aim #5: To measure acute or chronic effects of gastric bypass operation on meal size, meal number, body weight and body composition via carcass analysis.

The above specific aims, will provide a comprehensive, focused approach to the key control points in testing our hypothesis as to how gastric stapling is effective in: i) reducing food intake and body weight loss, and ii) giving an indication as to which body compartment contributes relatively most to the weight loss. An understanding of the neurobiological control points influencing the hyperphagia of obesity, and its reversal, will permit the rational development of medical modalities to ameliorate obesity. As outlined in **Section D. Research Design and Methods,** a series of studies have been designed in which overlapping aspect of each specific aim are tested, but not necessarily in the order shown in **Figure 2**.

SECTION B. BACKGROUND AND SIGNIFICANCE

Obesity and Its Complications: Using body mass index as the criterion of obesity, it is estimated that 25%-33% of the United States population is overweight, while another 30% are obese (14). Obesity is associated with increased incidence of cholelithiasis, adult onset diabetes mellitus, hypercholesterolemia, CHD, hypertension, and stroke; obesity complicates their clinical management as well. Obesity significantly contributes to osteoarthritis and other orthopedic joint problems (back, hips, and feet) as well as to increasing the frequency of prostate, colorectal and ovarian cancer (15). A weight increase of 20% above ideal body

weight results in about a 20% decrease in life expectancy. The current annual health care costs of obesity is estimated to be 40 billion and projected to increase to 70 billion as the population increases (16).

Diets may be temporarily successful in a limited number of patients, but rarely help to maintain long-term weight loss. Other forms of behavior modification have yielded similar poor result (17) while current pharmacological approaches either do not work well or have unacceptable side effects (18,19). Morbid obesity is a crippling disease, which costs billions of dollars in health care, and causes much subjective human misery.

Because all types of surgical procedures for morbid obesity have approximately 20% long-term complication rates, including anastomotic strictures, liver failure, nephrolithiasis, chronic electrolyte abnormalities and persistent diarrhea and operative mortality due primarily to cardiovascular complications, effective long-lasting non-surgical treatment of morbid obesity is urgently needed. It must be based on a full understanding of the pathophysiological factors regulating meal size, meal number and food intake at a cellular level, which promises the key to effective treatment.

2.Development and Progression of Gastric Bypass Procedure: In 1966, Mason began restrictive gastric surgery for morbid obesity with the gastric bypass, using a loop gastrojejunostomy (20,21). The limited storage capacity of the proximal gastric segment and the narrow outlet produced early satiety. Over distention caused distress and vomiting, promoting a change in eating behavior. Studies showed the effectiveness and safety of the loop gastric bypass. Effective features included the following: (a) ideal pouch size 50 ml or less, in order to assist weight loss and include acid-secreting mucosa in the distal segment to avoid marginal ulcer (22-24), (b) low serum gastrin levels (22-25) and (c) optimal diameter of the gastrojejunostomy anastomosis being 12 mm (22). In 1977, John Alden introduced the use of a 90-mm automatic stapling device to cross-staple the stomach in continuity without transsection (26). Ward Griffen modified Mason's divided-stomach loop gastric bypass by performing a Roux-en-Y gastrojejunostomy (27, **Fig. 3**). In 1978, the most common gastric operation became the undivided gastric bypass with Roux-en-Y gastrojejunostomy (**Fig. 4,** 28). Stapling techniques were then variously applied to the performance of the Roux-en-Y gastric bypass. Weight loss is inversely related to pouch size (29). Reported operative mortality was <1% and morbidity 4% (28,30), and more than 90% of patients had lost more than 50% of excess weight at 3 years (28,30,31). We have validated and repeatedly reproduced this operation in the rat, as documented in **SECTION C. PRELIMINARY STUDIES.**

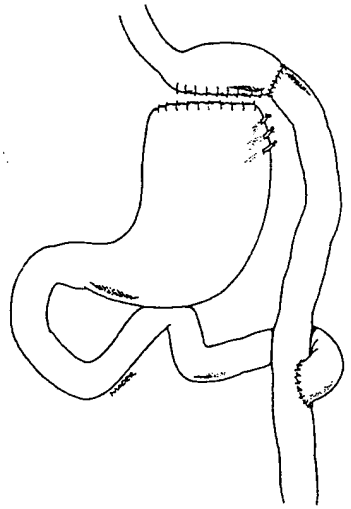


Fig. 3. Mason-Griffen divided- stomach, with 60 ml proximal segment.

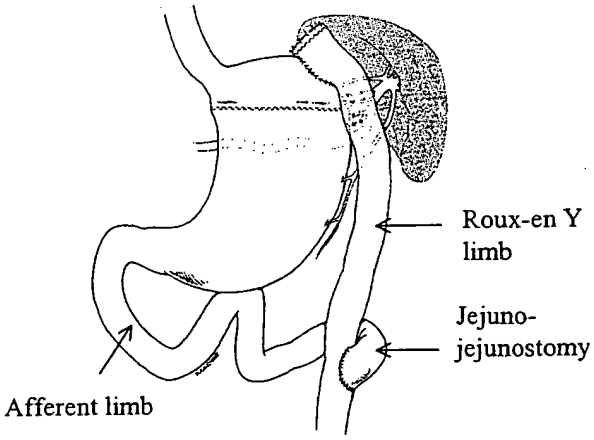


Fig. 4. Subsequent Roux-en-Y stapled undivided gastric bypass.

3. Relevance of Ghrelin: Ghrelin is a peptide/neuropeptide released from the stomach in response to fasting, which stimulates food intake. It is an endogenous ligand for the growth hormone secretagogue (GHS) receptor,

isolated in1999 by Kojima and Kangawa (4). The purified ligand is a 28 amino acid peptide, which they termed " ghrelin " derived from "ghre" is the Proto-Indo-European root of the word "growth". Ghrelin and its mRNA are also present in the hypothalamus, and GHS receptor are also expressed in hypothalamus. Both the peripheral and central administration of ghrelin stimulated food intake and increased body weight in freely feeding mice and rats, and growth hormone-deficient dwarf rats (7). Ghrelin concentrations in blood and mRNA in stomach increase by fasting and decreasing by refeeding (6-8). The ingestion of sugar, but not stomach distension decreases circulating ghrelin concentration (8). Anti-ghrelin antibody inhibits starvation-induced as well as dark phase food intake after peripheral and central administration in rodents. These data suggest that the presence of endogenous ghrelin stimulate the hypothalamus. These results indicate that ghrelin is the first appetite stimulatory peptide produced in the stomach which act as a neuropeptide in the hypothalamus.

4.Interrelationship of Ghrelin to Other Neuropeptides: GHS receptor mRNA is expressed at high levels in the arcuate nucleus (ARC) and the ventromedial hypothalamic nucleus (VMH). As summarized in a recent review by Inui (7), the peripheral administration of ghrelin or GHS receptor ligand induces ARC neurons to increase their electrical activity and to express c-Fos. But, neurons of VMN and other hypothalamic or forebrain nuclei were not similarly stimulated. About 50 % of these Fos-expressing neurons contain NPY mRNA. The GHS receptor mRNA was found to be expressed in 94% of ARC neurons that expressed NPY. Ghrelin increased both NPY and AGRP mRNA expression. Ghrelin-induced feeding was inhibited by pretreatment with anti-NPY or with anti-AGRP antibody or by the use of a specific NPY or AGRP antagonist.

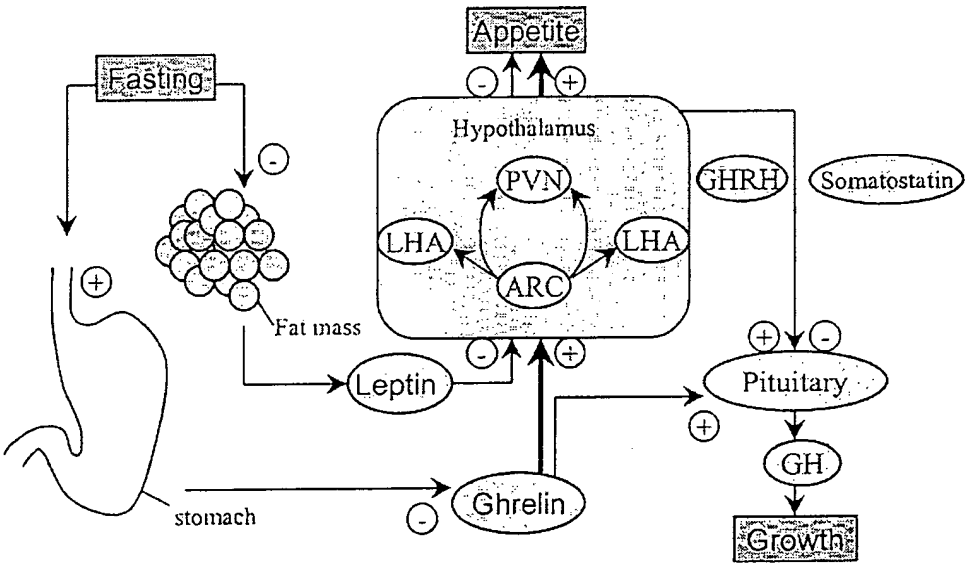


Fig. 5. A simplified model of the action of ghrelin and leptin on feeding-regulatory circuitry (7). Ghrelin is released from the stomach and acts as an orexigenic molecule. Fasting decreases leptin and increases ghrelin production, leading to orexigenic pathway.

These results indicate that the orexigenic effect of ghrelin is mediated by its action on the output of the arcuate NPY/AGRP neurons, as shown schematically in **Figure 5**.

5. Hypothalamic Control of Food Intake: Hypothalamic contribution to food intake control and energy metabolism regulation has been repeatedly confirmed since 1940. Control of meal size, meal number and food intake involves a complex biochemical interactions between peripheral organs and the brain, leading to the appropriate behavioral response in which learned hedonic aspects of food (presentation, smell, taste and texture) play a contributory role. Under normal circumstances daily food intake is under the control of the hypothalamus via a complex network involving a series of anatomically and functionally related foci receiving afferent input from the periphery and modulating the efferent biological response. In the hypothalamus different areas and nuclei have been implicated in the control of food intake including: the paraventricular nucleus, the

arcuate nucleus, the dorsomedial hypothalamic nucleus, the ventromedial nucleus of the hypothalamus (VMN) and the lateral hypothalamic area (LHA), among others (13).

Central food intake regulation involves complex relationships between neuropeptides, monoamines and other brain messengers and essentially occurs in the hypothalamus via the potent modulatory influence on food intake of the lower brainstem nuclei (32). Food intake (FI) consists of meal size (MZ) multiplied by meal number (MN) [FI=MZ x MN] which constitute a feeding pattern (33). Separate analysis of each component as related to change in neurochemical messengers, reflects the physiological significance of meal size as related to short-term satiation and meal number as related to long-term satiety. Any variable, which affects food intake, must do so via one or both of these indices. How these indices are affected by specific variables reveals useful information concerning the mechanisms responsible for the regulation of food intake.

We recognize that beside the monoamine neurotransmitters ie. dopamine and serotonin, many other chemical messengers are involved in the central regulation of food intake and also have their receptors in the hypothalamus. Among them other monoamines eg GABA, norepinephrine and epinephrine; neuropeptides eg Orexin, NPY, CRF, melanocyte concentrating hormone, cytokines, hormones eg CCK, leptin, insulin, glucagon, estrogen and testosterone. Thus their interaction can potentially influence feeding activity.

6. Dopamine (DA) and Serotonin (5-HT) in Obesity: DA and 5-HT are two critical food intake related neurotransmitters. The absence of DA production in the knockout mouse, which lacks tyrosine hydroxylase expression (34), causes inability to initiate feeding. This can be restored by tyrosine hydroxylase gene delivery into the striatum (35). Hence, DA has been thought to be required to initiate each meal, and thus can be associated with meal number. Increased dopamine concentrations in the VMN, the area of neuroendocrine and autonomic regulation of metabolism, accompanies food intake. Medial hypothalamic lesions produce both hyperphagia and morbid obesity, suggesting that VMN dopamine is involved in regulating both food intake and the body weight regulation. Numerous studies characterize 5-HT and the serotonergic system in the inhibitory role of food intake. The suggestion that 5-HT acts to inhibit food intake is based on findings that a variety of different direct and indirect acting agonists at central 5-HT synapses cause dramatic reduction in food intake. Evidence exists that DA and 5-HT-releases are interrelated (36). The evidence that DA and 5-HT are important in the hypothalamic regulation of feeding and contribute to the development of obesity is based on a series of studies. i) injections of monoamines, their antagonists or their agonists into the hypothalamus produces changes in food intake and in meal size/meal number; ii) in vivo microdialysis detects acute changes in monoamine concentrations in the hypothalamus in relationship to food intake and its components; iii) hypothalamic monoamine concentrations differ in obese and lean Zucker rat phenotypes (37-43).

7. Hypothalamic Dopamine Receptors in Obese Zucker Rat: The ventromedial hypothalamus (VMH), the lateral hypothalamic area (LHA) and other hypothalamic nuclei are centers for the regulation of metabolism (for review see Schwartz et al. [44]), via the potent modulatory influence on food intake of the lower brainstem nuclei (32). Daily food intake is a function of meal size (MZ) and meal number (MN) [FI=MZxMN], which constitute feeding pattern (33). The rat on *ad libitum* diet is able to perfectly adjust meal number to meal size in order to maintain constancy of daily food intake, suggesting an existence of neurochemical mechanisms responsible for sensing and measuring both, meal size and postmeal interval. The obese Zucker rat is not an exception of this rule and displays a feeding pattern characteristic for obesity with large meal size and low meal number. Experimental manipulation in the hypothalamus, by increasing either dopamine or serotonin via pharmacological infusions or via fetal cell transplants induce changes in food intake, and also lead to changes in feeding pattern (37,38). Such data provide the evidence that changes in meal size and number are appropriate to the necessary metabolic circumstances. For instance, for an efficient control of body weight and stimulation of energy expenditure it is advisable to increase meal number and reduce meal size (45). Thus, by understanding the mechanisms of induction large meal sizes it would be possible to find an alternative

therapeutical strategy for obesity treatment. Since normal functioning of dopaminergic neurotransmission has been shown to be indispensable in initiating food intake and for feeding and for survival (46), any abnormality involving the dopaminergic system is reflected by altered feeding behavior. Via in vivo microdialysis, we showed that in the normal rat, the release of dopamine (DA) in the LHA and in the VMH correlates with meal size and post meal intervals i.e,. meal number (47,48) while in the obese Zucker rat the ingestion of food is accompanied by an exaggerated release of dopamine in these two brain areas (39,49). Thus, in the obese Zucker rat, food intake-accompanied hypothalamic release of DA is qualitatively different from that in the lean Zucker rat indicating an abnormal dopaminergic neurotransmission. Dopamine acts on its specific receptors belonging to the G-protein coupled receptor family which are classified into two subgroups: D1–like and D2–like, according to their function to stimulate or to inhibit adenylyl cyclase respectively (for review see ref 50). Normal functioning of dopaminergic signaling is tightly dependent on the level of expression of dopaminergic receptors (for review sees ref 51). The status of the postsynaptic dopaminergic system in the hypothalamus during obesity as these relate to hyperphagia was investigated and these data are shown in **SECTION C. PRELIMINARY STUDIES.**

8.Relationship Between Aminergic and Peptidergic Neuropeptides: A close functional and anatomical relationship exists between the aminergic and peptidergic neurotransmitters in the regulation of food intake, at different levels of the mid-brain and hypothalamus. The precise relationship and their relative function(s), remain speculative i.e., "Which is the horse and which is the cart". A functional example can be gained from knock out models, which suggest that tyrosine hydroxylase (TH) knockout mouse model, lacks dopamine and exhibits aphagia and weight loss leading to death (34). The normalization of dopamine metabolism after TH gene delivery into the striatum restores food intake and weight gain (35,46). In contrast, an NPY knockout mouse exhibits normal food intake and normal growth (52). Immunocytochemical examples from our laboratory, demonstrating the coexistence in the same neuron of a peptide (NPY) and a classic small molecule neurotransmitter (5HT) in different areas of the brain, under different nutrient physiological conditions are shown in **SECTION C. PRELIMINARY STUDIES**. This raises the question as to whether one neuron can utilize more than one transmitter and the composition of chemicals at the end of the synapse. Some of these questions will be addressed by our proposed agonist and antagonist experiments.

9. Measuring Meal Size and Meal Number and their Relationship: Despite the extraordinary complexity of both the anatomy and the functions of the hypothalamic areas which regulating food intake, the outcome of these complex interactions may be expressed by a simple formula reflecting integrative behavior of food intake. Daily food intake (FI) is a function of meal size (MZ; reflecting short-term satiety) and meal number (MN; reflecting long-term satiety). Hence, FI f MZ x MN, or more simply, FI = MZ x MN. These are measured in the rat in real time by the use of the **Automated Computerized Rat Eater Meter**, developed in this laboratory **(see Methods).**

Meal number and meal size reflect two different physiological states and thus should have different neurochemical basis and different neuroanatomical (hypothalamic) areas. This basic concept helps to understand mechanisms controlling food intake in hyperphagia of morbid obesity. The equation FI=MN x MZ has a great practical impact, since under normal physiological conditions a change in one is offset by a compensatory change in the other, to maintain the constancy of food intake (33). We have repeatedly demonstrated this reciprocal relationship between meal number and meal size, to maintain the homeostasis of food intake under a series of different experimental conditions (For a review see ref 13). To function as compensatory mechanisms, it is likely that meal size and meal number are independently regulated in a way analogous to the reciprocal innovation controlling spinal reflexes. Moreover, it is likely that they are regulated in different but connected anatomical sites of the hypothalamus. Thus for the microdialysis studies we focus our attention on the lateral hypothalamic area (LHA) and the ventromedial nucleus of hypothalamus (VMN), whose anatomical links and functional reciprocity are established We studied intra-LHA and intra-

VMN changes in dopamine and serotonin neurotransmitter levels as they relate to the relationship to meal size and meal number (for a summary review see 13).

10. Relevance of the Zucker Rat as an Animal Model of Human Obesity: We selected the Zucker rat, because its biochemistry as an animal model of human obesity is well defined and is described in greater details in. The origin of obesity in the Zucker rat is a mis-sense mutation of the gene coding for leptin receptor. (53,54). This diminishes the leptin signaling to the brain (55) leading to numerous adaptive changes down steam of leptin target cells of the central regulatory systems which develop the Zucker phenotype, characterized by hyperphagia, large meal sizes, fewer meal numbers, positive energy balance and obesity. For a succinct review of a lengthy treatise on this subject encompassing more than 75 references, the referee is encouraged to refer to reference 56.

However, obese Zucker rats do not show complete absence of leptin action in intracellular signal transduction as expected, but a reduction of signal transduction associated with leptin. Thus, even the altered leptin-signaling pathway in the obese Zucker rat, due to a genetic mutation results in leptin resistance, this reflects the leptin resistance also observed in human obesity; considered as polygenic. Although a rare case of leptin receptor mutation in obese humans has been reported, in randomly chosen obese humans displaying leptin resistance, no mutation of leptin receptor associated with obesity has been found. The chronic adaptation to the altered leptin signaling pathway in Zucker rats creates the Zucker syndrome of obesity which possesses all characteristic of human obesity, including: diabetes, insulin resistance, hypertension and renal failure. In this scenario, the down steam neuronal pathways activated or inhibited by leptin and involved in the regulation of food intake and energy balance, represent an important, if not, the most important morpho-functional structures in the pathogenesis of obesity. In our hypothesis, these, mostly peptidergic hypothalamic systems, undergo regulatory influence from autonomic nervous system and can be regulated by monoaminergic neurotransmitters (including dopamine and serotonin) to produce a similar metabolic output due to altered leptin signaling pathway. A further advantages to us and thus to this proposal in using the Zucker rat is that: i. this model has been extensively studied in our laboratory and thus we have a large data reference base (38,40-43,57,58) and ii. because it has a lean phenotype which serves as its control (38,40-43,57,58).

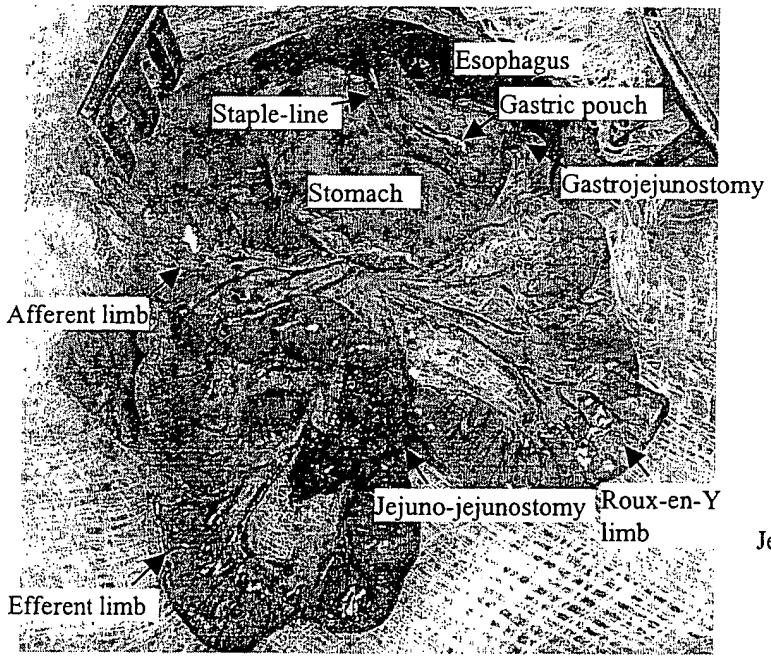
SIGNIFICANCE: As stated in the **SECTION A. SPECIFIC AIMS** and detailed in **SECTION B. BACKGROUND AND SIGNIFICANCE,** a significant proportion of the population is obese and the percent is increasing. As a nation we are facing an epidemic of obesity, starting in childhood and progressing to morbid obesity in adult hood. The health complications of obesity are also well recognized and add a significant health cost burden to our national annual budget. With the limited effectiveness of diets, pharmacological agents and behavior modification, surgery is frequently reverted to, particularly in life threatening complications of morbid obesity. The most popular operation performed is the Gastric Bypass and Roux en Y procedure, which has evolved over the years as encompassing the most practical and physiological approach to long –term weight loss, with the least horrific complications. Because this procedure can be done laparoscopically, and thus via minimal invasiveness, it is being performed in increasing numbers through out the US and Europe. How it works remains speculative. We propose to investigate its mechanism of action, having developed a reliable and reproducible rat model, as out lined in our **Hypothesis.** Our preliminary data indicate that our line of investigation will be fruitful and merits your support.

SECTION C. PRELIMINARY STUDIES The experiments below are not presented as being in anyway definitive but as indications that i) our methods are suitable for the proposed work, and ii) the postulated results are likely to be forthcoming when the needed studies or additional repetitions are completed.

To develop a dependable rat-Gastric Bypass Roux-en-Y Model model, we initially used the less expensive Sprague Dawley species. After obtaining permission from the Committee for the Humane Use of Animals we operated (open technique) on a series of rats to work out the technical problems inherent in creating a small

gastric pouch via gastric stapling method, a hand-sewn gastro-jejunostomy, and a hand-sewn jejuno-jejunostomy etc, as outlined in **SECTION B. BACKGROUND AND SIGNIFICANCE.** Other challenges successfully overcome included, post-operative management, feeding with liquid diet **(see Methods)** and then with regular coarsely ground rat chow **(see Methods)** and profiling daily food intake and body weight changes. To this was added time points at which rats were killed to obtain biochemical data, and to perform quality control necropsies. The procedure were performed by the same "surgical team", led by Dr Xu (a research fellow with general surgery credentials; see **Biographical Sketch**), assisted by Tomoko Tada MS my laboratory technician, and Dr. Ohinata, the Co-Principal Investigator. After a relatively short but steep learning curve, the operation became standardized, lasting 75 min, and with 100% success. At this stage a switch was made to the Zucker rat. The rationale for using the Zucker rat as a model of human obesity is summarized in **SECTION B. BACKGROUND AND SIGNIFICANCE.** It is anticipated that the same "surgical team" will continue to function together for the tenure of the application. We have done selected studies during the post-gastric bypassperiod, as a guide to the relevance of our hypothesis. Additional studies in all specific aims need to be done to confirm our hypothesis and to develop rational therapeutic modalities.

1.Developing a Rat Gastric Bypass-Roux-en-Y Model: Adult rats were purchased. Rats were housed in holding wire mesh cages for 1 week after purchase to acclimate them to the constant study en conditions: 12-h light cycle (06.00-18.00 h), 26±1°C room temperature, 45% humidity. They standard rat chow (Diet 5008; Ralston Purina, St. Louis, MO). Food and tap water were availa Daily food intake and body weight was measured. Rats were randomly divided into gastric by operation groups. Rats were food deprived overnight, and after Ketamine and Xylazine anesth abdomen shaved, prepped and draped aseptically. For the rats undergoing the Gastric Bypass a gastric operation, the abdomen was opened through the mid-line and the stomach and distal es The gastric fundus was cross-stapled in continuity without transsection with a two-row staple (a pouch consisting 20-25% of total stomach size. Then an end to side gastro-jejunostomy (G-J jejunostomy (J-J) was hand-sewn. The diameter of the G-J anastomosis was 4-5 mm and that J-J anastomosis was 8-10 mm in diameter. The length of the afferent limb of jejunum was 15-1 ligament of Treitz and Roux-en-Y limb of jejunum was 10 cm long. The final anatomical and f arrangement is similar to that created in humans, and is shown schematically and via photograp



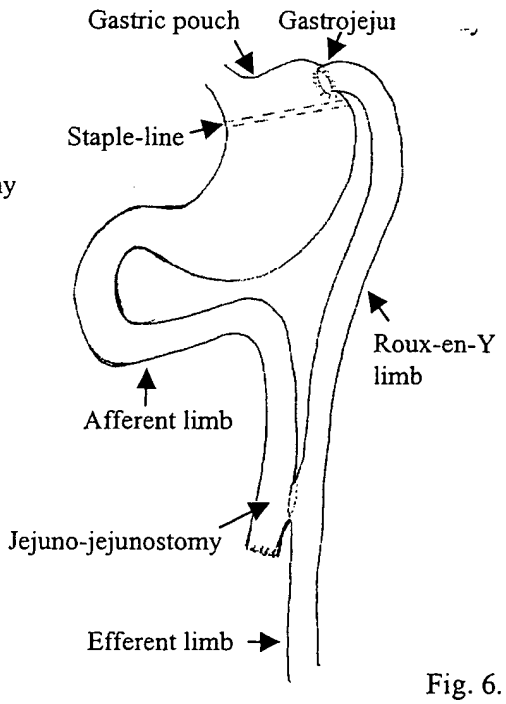


Fig. 6.

Rats in control group underwent a sham operation, which consisted of opening the abdomen, mobilizing the lesser and great curvature, laying out 15-20 cm of jejunum, and after replacing the contents, closing the abdomen. All rats were fasted for 24 h post-operatively, then offered liquid food ad lib for the first 7 days (Boost, 1 kcal/ml; Mead Johnson, Evansville, IN, **see Methods**). Thereafter the diet was changed to solid food food for 14 days (Purina Chow #5008, 3.5 kcal/g, **see Methods**). After operation rats are injected with normal saline, 15-20 ml/day, for the first 1-3 days, if their oral intake was not considered sufficient, to prevent dehydration. Thereafter, rats were sacrificed and underwent necropsy. The sizes of gastric pouch was measured and the volumes of pouch calculated using the formula for a prolate spheroid (volume=1/2 ab^2, where a is the longer and b is the shorter dimension).

2.Post-Gastric Bypass Food Intake and Body weight Changes: After gastric bypass operation, **Figure 7** shows the body weight change after 7 days on a liquid diet, in a limited number of obese Zucker operated rats (closed diamond) and their obese sham pair-fed control (open diamond). Both groups lost significant weight after the operation. The initial, day 1 weight gain was due to sub-cutaneous hydration. The lean Zucker control rats (closed triangle), in contrast did not lose weight. Their sham operated pair-fed lean Zucker rats(open triangles) lost weight, as expected.

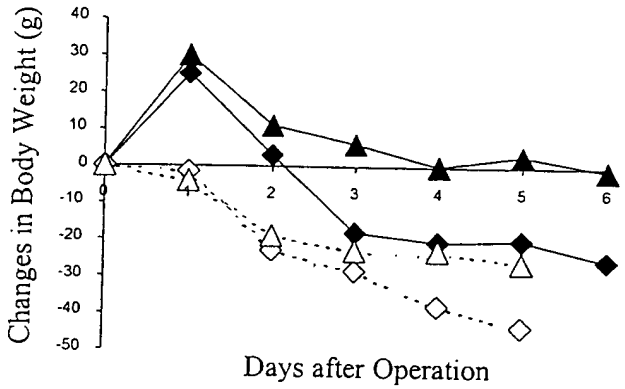


Fig. 7. Changes in Body Weight after Gastric Bypass Operation in Zucker Rats.

3.Expression of Ghrelin mRNA: To test whether afferent feeding-stimulatory signal was decrease after gastric bypass operation, we measured the ghrelin mRNA in stomach one week after the operation in non-food-deprived Sprague Dawley rats using RT-PCR (polymerase chain reaction after reverse transcription of RNA) analysis, as detailed in **Method Section.** The bands of 296 and 249 base pairs corresponded to the expected fragment of ghrelin and β-actin, respectively **(Figure 8)**. Ghrelin mRNA expression was decreased after the operation as compared to control, suggesting that early satiety signal from stomach occurred after the gastric bypass operation.

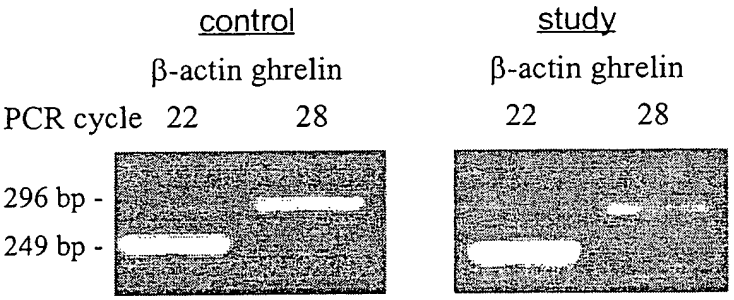


Fig. 8. RT-PCR for Ghrelin mRNA from stomach one week after gastric bypass operation in SD rats. The bands of 296 and 249 base pairs corresponded to the expected fragments of ghrelin and β-actin, respectively.

3. Blood glucose, triglyceride and free fatty acid FFA): Blood glucose, triglyceride and FFA concentrations one week after the operation from both Sprague Dawley (SD) together with their control and lean and obese Zucker rats are shown in **Figure 9** below. Blood glucose was not affected by the operation in SD nor in either Zucker rats. But, triglyceride levels after the operation were decreased in SD and obese Zucker rats as compared to ad lib and pair-fed control, respectively. Because of the malabsorption nature of the operation, lipid absorption is likely to be decreased. And, because of the decrease in food intake secondary to the small gastric pouch and the resulting putative early satiety an increase in triglyceride utilization occurs after the operation. FFA levels were decreased even in Zucker rats as compared with pair-fed control, suggesting that the utilization of FFA is increased after the operation, contributing to the weight loss ascribed to the operation.

Since, during starvation, FFA is synthesizes in adipose tissue and increased by activation of hormone sensitive lipase, the gastric bypass operation differs metabolically from normal starvation, as proven by the pair–fed control data. In the light of ghrelin's known activity to induce a positive energy balance, our finding of the down regulation of ghrelin after gastric bypass was expected to decrease the utilizations of FFA and triglyceride. Instead our findings of an increase in FFA utilization, in conjunction with the upregulation of mRNA ghrelin after gastric bypass provides compelling and tantalizing preliminary evidence that gastric stapling operation has its effect via ghrelin. However, the complex interaction of this peptide together with aminergic (DA and 5HT) neurotransmitters in food intake related hypothalamic nuclei, necessitates further studies, as outlined in **SECTION D. RESEARCH DESIGN AND METHODS.**

4.Carcass Analysis: In a recently completed study, we continuously infused serotonin intra-peritoneally via mini-osmotic pump for 14 days in obese and lean Zucker rats and their controls, while measuring daily food intake, body weight change and on sacrifice, performing carcass analysis The purpose was to inhibit food intake. **Figure 10** below shows the changes in body weight and body fat. The infusion of serotonin decreased food intake significantly in the lean rats. It did not influence obese Zucker as much as lean Zucker, which showed a significant decrease in body weight, as fat. The resistance by obese rats to increase energy output during serotonin infusion show a primary defect in post synaptic serotonergic neurotransmission in the VMN of obese rats, which can contribute to the pathogenesis of Zucker syndrome of obesity.

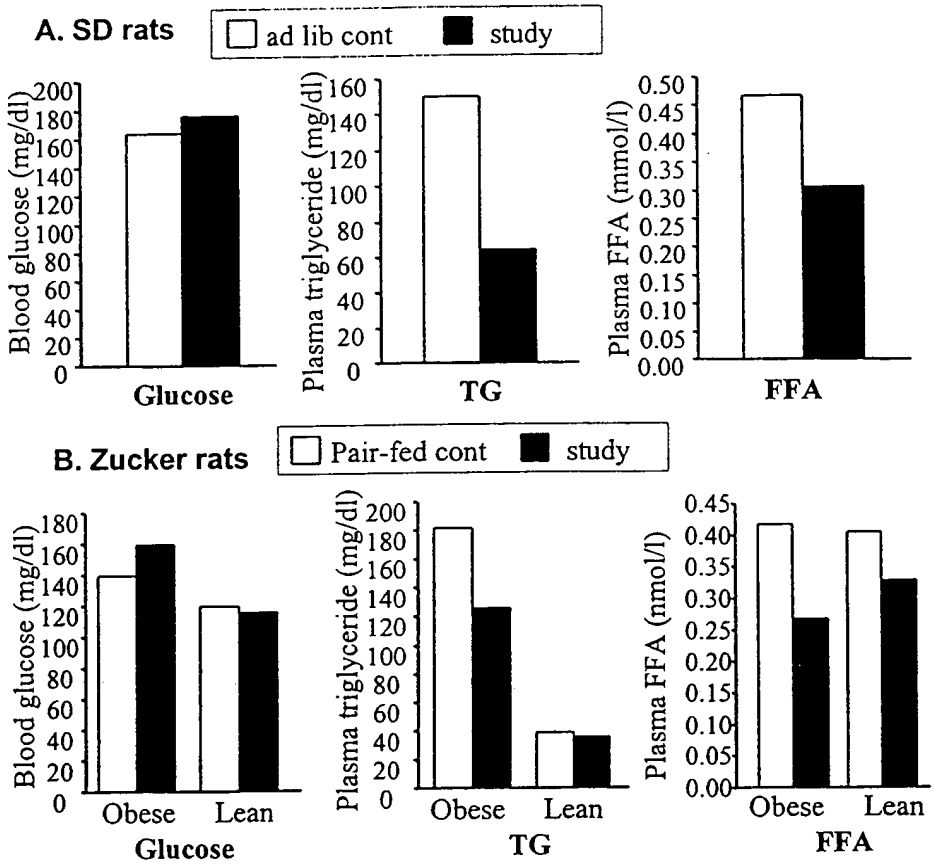


Fig. 9. Effects of gastric bypass operation on blood glucose, triglyceride and FFA one week after operation in SD (A, n=1) and Zucker (B, n=1-2) rats. Numbers in parenthesis show the number of rats.

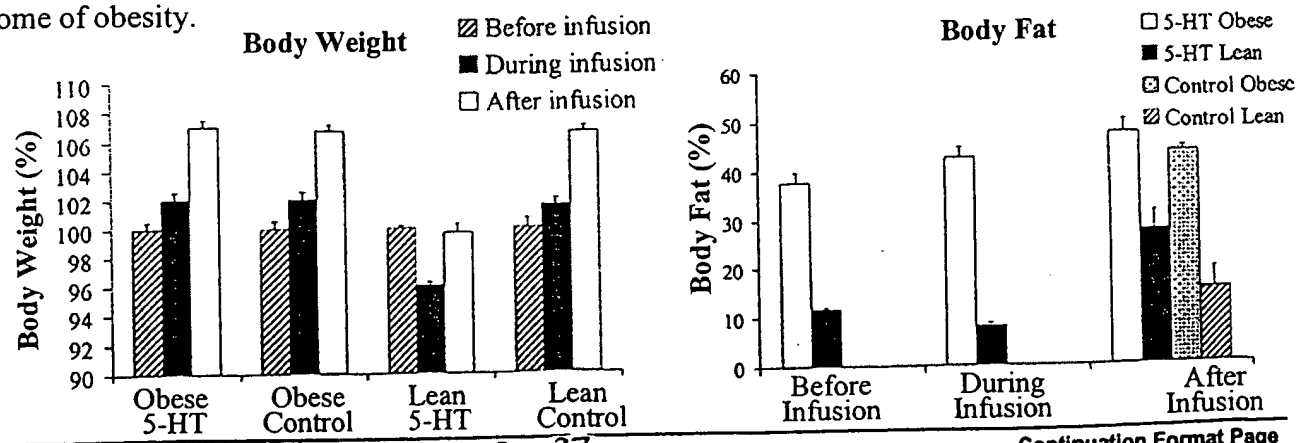
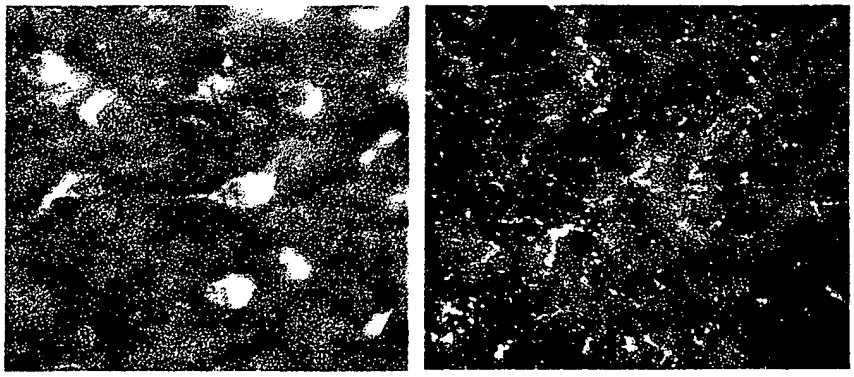


Fig. 10.

5. Expression Of Dopamine Receptors in Hypothalamus of Obese Zucker Rats: In our previous microdialysis studies, basal and food intake accompanied dopamine release was significantly higher in the hypothalamus of obese vs. lean Zucker rats (39). Based on these observations we determined whether the postsynaptic dopaminergic system is also compromised in obesity. Dopamine D1 and D2 receptor mRNA expression was studied in the ventromedial hypothalamus (VMH), lateral hypothalamic area (LHA) and the adenohypophysis (AH) of obese and lean Zucker rats using RT-PCR technique. In obese Zucker rats we found an up-regulation of D1 receptor mRNA in the VMH and AH, and a down-regulation in the LHA, while D2 receptor mRNA was down-regulated in both the VMH and LHA, but not changed in the AH, as compared to lean rats. Also, an increase of D1 receptor staining was seen in the medial hypothalamic nuclei of obese rats by immunohistochemistry. We selected the VMH to test if the observed change in the DA receptor expression of obese rats induce behavioral sensitization to as expressed by hyperphagia. The overnight food deprived rats received a single injection (10 nmol) of Sulpiride (D2 receptor antagonist) or saline as control, then food was provided and one-hour food intake was measured. Food intake after Sulpiride vs. Saline injection was greater in obese rats but was not different in lean rats. Our data suggest that down-regulation of D2 receptor in the hypothalamus and particularly in its medial part, may initiate a number of pathophysiological events, which will ultimately lead to the development of obesity. These would include an exaggerated dopamine release during food ingestion, which will induce behavior sensitization for having large meals, which in turn are propitious for development of obesity. High level of D1 receptor expression in the VMH and low in the LHA may also contribute to the specific feeding pattern in obese rats represented by large meal size and low meal number (58)

6. NPY Innervation of Hypothalamic Neurons Expressing 5-HT (1B) Receptors: The hypothalamus is heavy innervated by neuropeptide Y (NPY) fibers originating from both hypothalamic arcuate nuclei and brain stem neurons (59,60). Food intake is also under the control of the serotoninergic system via serotonin 5-HT (1B) receptors (61,62). In the absence of data concerning its distribution in the brain in general, and in the hypothalamus in particular, we compared distribution of hypothalamic neurons expressing 5-HT(1B) receptors in normal Fischer rat brain in comparison to NPY innervation to determine the sites and frequency of possible co-existance or co-localization of NPY and serotonin in the hypothalamus. Rats were trans-cardially perfused with saline, followed by 4% paraformaldehyde. Immunocytochemical labeling for NPY and 5-HT(1B) receptors was performed on the parallel series of hypothalamic coronal sections. Using peroxidase-antiperoxidase monolabeling with specific polyclonal antibodies to NPY and fluorescent technique, the most dense fiber and terminal network for NPY is localized in paraventricular (PV), arcuate nuclei (ARC) and periventricular region. Prominent NPY fiber systems were also visualized in dorsal, lateral and ventrolateral hypothalamus. The distribution of neurons which expressed 5-HT(1B) receptors was analyzed using specific polyclonal antibodies. Immunostaining revealed an abundance of neurons with 5-HT_{1B} reaction in the cytoplasm of cell bodies and in the proximal dendrites. 5-HT_{1B} immunoreactive neurons were highly specifically distributed in

Figure 11. Immunofluorescent Double-Labeling.
NPY fibers and Terminals Surrounding Hypothalamic Neurons
Expressing 5-HT_{1B} Receptors



Paraventricular nucleus:	Ventrolateral hypothalamus:
Red - NPY	Yellow - NPY
Yellow- 5-HT_{1B} receptors	Green – 5-HT_{1B} receptors

the hypothalamus and thalamus. The most intense IR was observed in the hypothalamic magnocellular nuclei (supraoptic, paraventricular, retrochiasmatic) and parvicellular arcuate nucleus. Prominent group of large and medial neurons with different 5-HT_{1B} staining pattern is located in the lateral hypothalamus with specific distribution in rostrocaudal direction. Medium and light staining was observed in neurons of anterior periventricular, ventromedial and dorsomedial hypothalamic nuclei. Comparison of NPY fibers (63) and 5-HT_{1B} immunoreactive neurons (64) in the hypothalamus reveal that the main places of their co-distribution are: paraventricular and supraoptic nuclei, perifornical and lateral hypothalamic regions. Double immunofluorescent labeling demonstrated dense network of NPY fibers and possible terminals around 5-HT_{1B} receptor - immunopositive neurons in the paraventricular nucleus and lateral hypothalamus **(Figure 11)**. These data show, that the hypothalamic neurons in the described areas are under the influence of both serotonin and NPY neuronal systems.

To test whether changes in food intake related to a change in NPY in the hypothalamus, we compared NPY expression between normal Fischer, as control, and a readily available model in the laboratory of decreased food intake, namely a tumor-bearing rat at the onset of anorexia. In the anorectic rat, with a significant decrease in food intake, the concentration of nerve fibers appeared to decrease significantly in the ventral part of the supraoptic nucleus which is particularly rich in the magnocellular vasopressinergic neurons. A decrease of the concentration of NPY immunoreactive fibers was also observed in the medial preoptic area just dorsal to the optic chiasm. Data suggest that a change in food intake, i.e., the decreases in food intake at the onset of cancer anorexia is related to modified NPY turnover and related neurotransmission in distal axons of the supraoptic nucleus and preoptic area, influencing food intake activity of anorexia. It seems a reasonable deduction that, when the converse preliminary experiment currently in progress is completed, we will observe an increase in NPY and thus in ARRP, and that after gastric bypass operation, a similar biochemical picture will be seen in the hypothalamus, was seen in the anorexia of cancer.

SECTION D. RESEARCH DESIGN AND METHODS

RESEARCH DESIGN: Overview of Commonalties to all Experiments. Some of these experiments will be carried out initially in Sprague Dawley rats to determine a normal data base, and then in an obese hyperphagic rat model. We have selected the Zucker rat, because its biochemistry is well documented, and has been extensively studied in this laboratory, and because it has a lean phenotype which serves as its control. After rats are purchased, they are placed into individual cages equipped with Automated Computerized Rat Eater Meter (ACREM) to measure food intake, meal size and meal number in study environment for acclimation, for two weeks. Each study requires that the (SD) and Zucker rats be randomly divided into three different experimental groups, identified as: Gastric bypass operated study group (**Gastric bypass**) and two controls, the i. Sham operated ad-lib food (**Sham ad lib**) and the ii. Sham operated pair-fed group (**Sham Pair-Fed**). **Figure 12** shows the general schema of the experimental design, based on data obtained in preliminary studies.

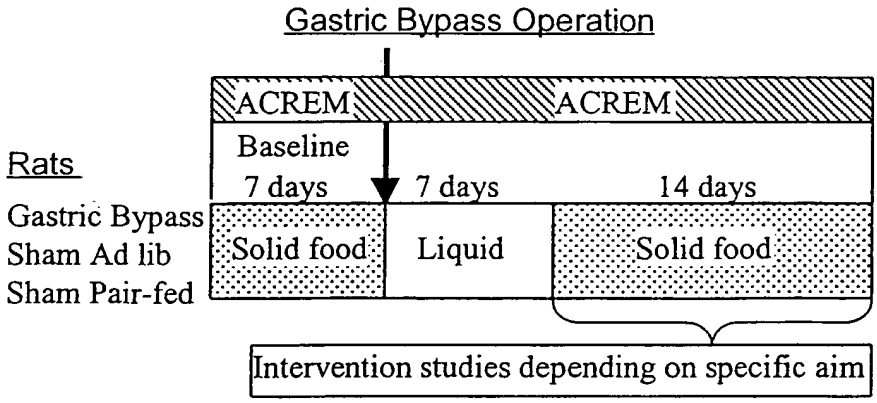


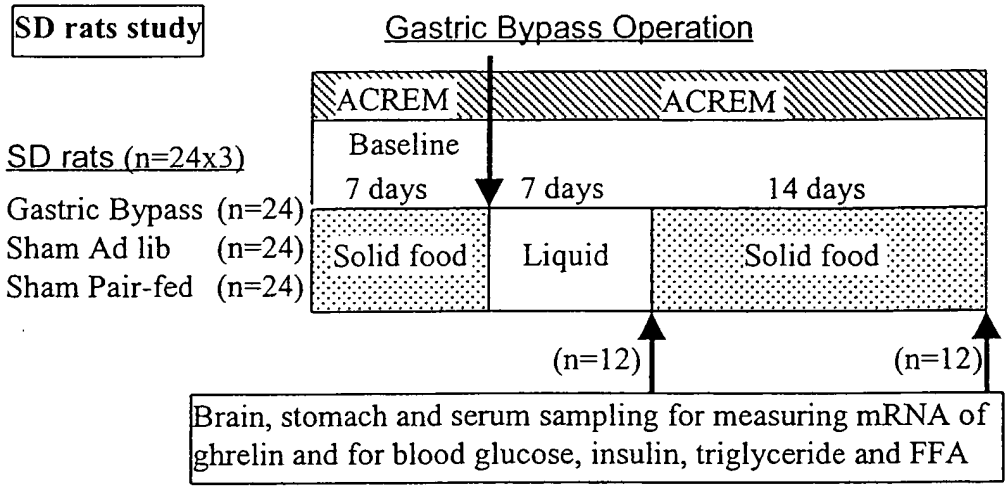
Fig. 12. The general schema of the experimental design

Rats are replaced into their individual ACREM cages for 7 days on solid rat chow (see **Methods** for detail). Then rats are subjected to operation (see **Methods** for detail). And replaced into their ACREM cages on a liquid diet (see **Methods** for detail) for 7days, followed by14 days of solid rat chow diet. After the period of

liquid diet and during the period of solid died a variety of biochemical, molecular biological and immunocytochemical studies will be done, depending on the specific aim of each study, as out lined below.

Experiment #1: The purpose of this experiment is to determine short- and long-term effect of gastric bypass on gastric ghrelin expression, on the feeding pattern and body weight change using the Automated Computerized Rat Eater Mater (ACREM), as outlined in **Figure 13** below, and in our specific aims.

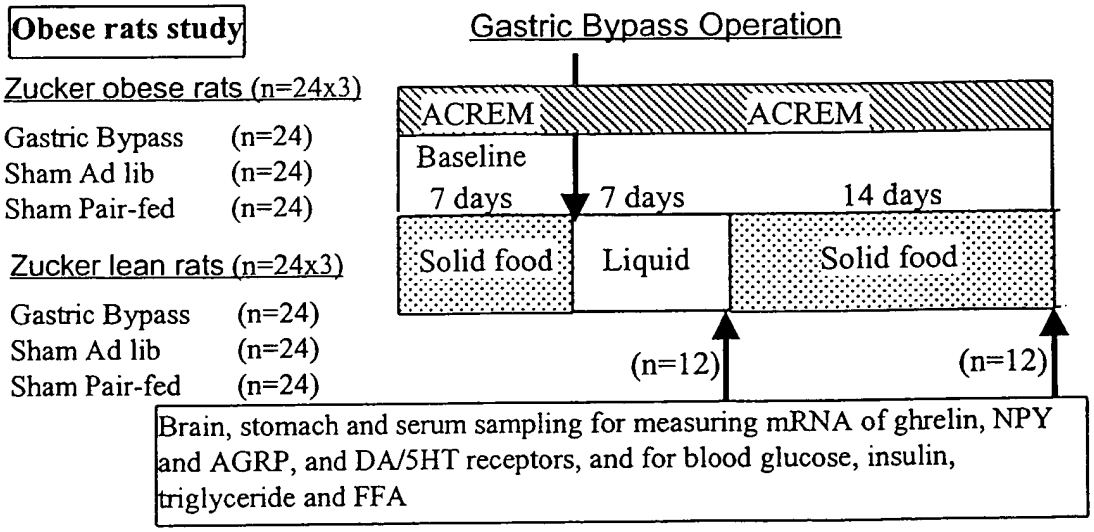
Figure 13: Experiment #1 for specific aim #1 and 5



After two week acclimation, SD rats (n=72 gastric bypass, or sham operated ad-lib food (Sham ad lib), Sham operated pair-fed group (Sham Pair-Fed) will be studied in ACREM to measure food intake, meal size and meal number (16 rats in each experiment). As depicted in schema, after one week of baseline data, rats will be operated for gastric bypass or sham operation, under ketamine anesthesia (see **Methods**). SD rats will be fed liquid food (see **Methods**) for one week after the operation to facilitate early recover from operative trauma, then, changed to solid rat chow (see **Methods**). Rats one or three weeks after the gastric bypass or sham operation will be killed by decapitation under anesthesia. Brain and stomach will be immediately removed, the hypothalamus and the gastric fundus will be dissected, and blood sample will simultaneously be taken. The ghrelin mRNA will be extracted from tissue homogenates and RT-PCR will be performed (see **Methods**). Gels will be scanned and analyzed by comparing with corresponding expression of β-actin in the same brain and stomach samples using Sigma Gel computer program (see **Methods**). In addition, estimates of peripheral energy metabolism ie. blood glucose, insulin, triglyceride and FFA will be measured using enzymatic colorimetric assays and carcass analysis performed to determine the component of boby composition contributing to weight loss (see **Methods**).

Experiment #2 (Zucker rats study): The purpose of this study is to investigate short- and long-term effects of gastric bypass operation on the feeding pattern, body weight and carcass composition changes. These parameters will be correlated to the hypothalamic biochemical changes in aminergic and peptidergic systems after gastric bypass operation in Zucker rats using RT-PCR methods as shown in Fig 14.

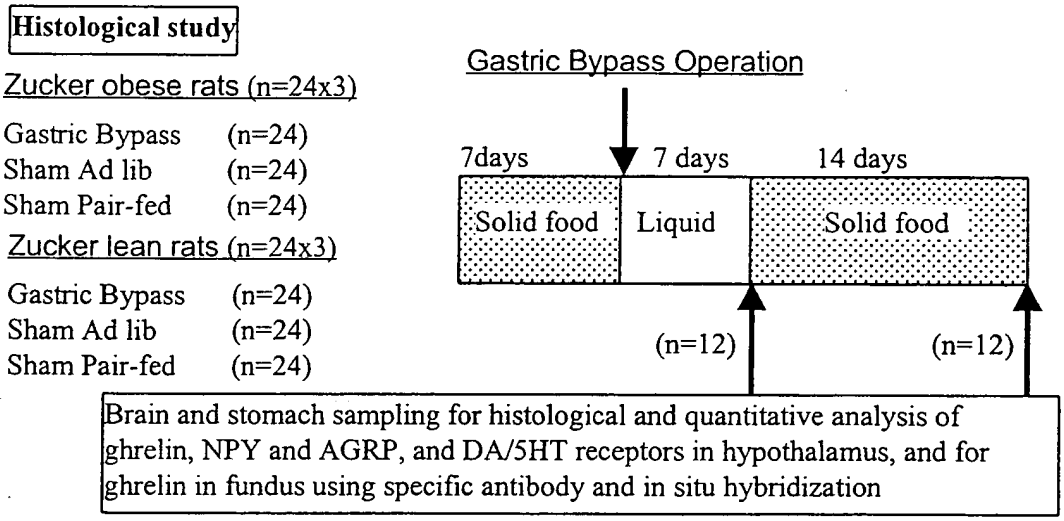
Figure 14: Experiment #2 for specific aim #1, 3, 4 and 5



Obese Zucker (N=72: gastric bypass n=24; or sham operated ad-lib food (Sham ad lib, n=24), or Sham operated pair-fed group (Sham Pair-Fed, n=24) and lean rats (n=72; gastric bypass, n=24, or sham operated ad-lib food (Sham ad lib, n=24), or Sham operated pair-fed group (Sham Pair-Fed, n=24) will be acclimated for two weeks and studied in ACREM to measure food intake, meal size and meal number. Then, after one week of baseline data, rats will be operated for gastric bypass or sham operation under the ketamine anesthesia **(see Methods)**. Zucker rats will be fed liquid diet **(see Methods)** for one week after the operation, then, changed to solid food. Rats one or three weeks after the gastric bypass or sham operation are killed by decapitation under anesthesia. Brain and stomach will be immediately removed, hypothalamus and fundus will be dissected, and blood sample will simultaneously be taken. The mRNA will be extracted from hypothalamus homogenates for measuring the expression of ghrelin, NPY or AGRP and of dopamine D1, D2, serotonin 5HT1B or 5HT2C receptors **(see Methods)**. Similarly, mRNA will be isolated from stomach to measure ghrelin expression. Then, RT-PCR will be performed, and PCR products will be scanned and analyzed by comparing with corresponding expression of β-actin using Sigma Gel **(see Methods)**. In addition, blood glucose, insulin, triglyceride and FFA will be measured using enzymatic colorimetric assay and carcass analysis will be performed **(see Methods).**

Experiment #3 (Histological study): In the previous experiment, Experiment #2, brain biochemical changes in aminergic and peptidergic systems are investigated after gastric bypass operation in Zucker rats using RT-PCR methods. However, we also need to confirm the specific sites and the amount of peptide and its gene expression, by using immunohistochemistry and in situ hybridization for: ghrelin, NPY, AGRP and dopamine D1, D2, serotonin 5HT1B or 5HT2C receptors in hypothalamus, and for ghrelin in stomach in Zucker rats.

Figre 15: Experiment #3 for specific aim #1, 3 and 4



After two weeks acclimation, Zucker obese (n=72) and lean (n=72) rats will be operated for gastric bypass or sham operation. Zucker rats will be fed liquid diet for one week, and then changed to solid food according to the schema. Non-food deprived rats will be anesthetized and perfused intracardially with paraformaldehyde for brain and stomach fixation one or three week after gastric bypass operation **(see Methods).** Brain and stomach will be sectioned on a cryostat and sections will undergo immunocytochemistry or in situ hybridization for ghrelin, NPY or AGRP and dopamine D1, D2, serotonin 5HT1B or 5HT2C receptors in hypothalamus, and for ghrelin in stomach. All processing of experimental and control material will be similar. Peroxidase-antiperoxidase (PAP) or Avidin-biotin (ABC) immunocytochemical methods will be used to prepare stable slides.

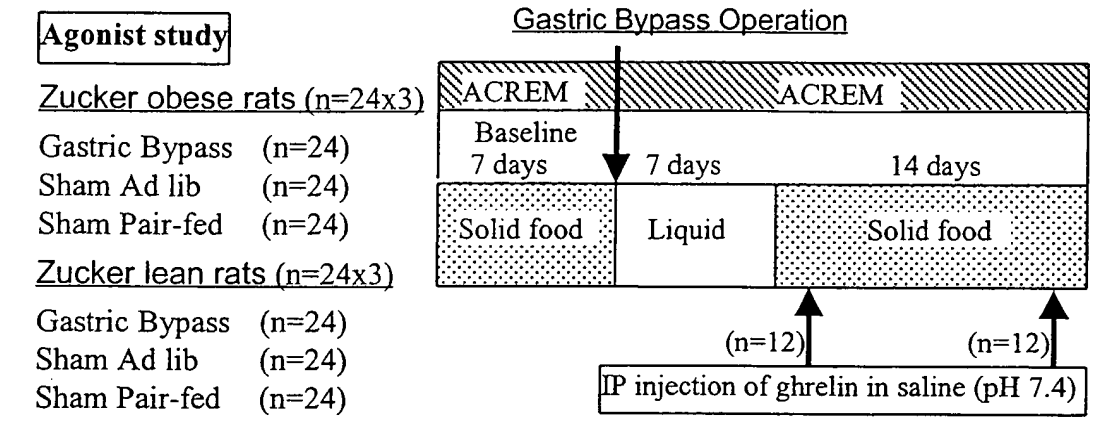
Slides will be examined for qualitative analysis in OPTON transmission brightfield microscope, then areas of interest will be photographed or digital images will be prepared using NIKON microscope. All images of the experimental and control material will be processed in the same way with Adobe Photoshop 6 computer program and than quantified by Image Pro computer program. This will give the possibility to quantify the

results of immunocytochemical reaction, and as a result changes in the receptor activity after operation in different hypothalamic structures.

Immunofluorescent doublelabeling with different labeling of secondary antibodies (FITC and TRITC) will be performed to analyze the possible colocalization of serotonine and dopamine receptors on the same neurons, and thus describe precisely the hypothalamic sites of serotonin/dopamine interaction involved in regulation feeding behavior

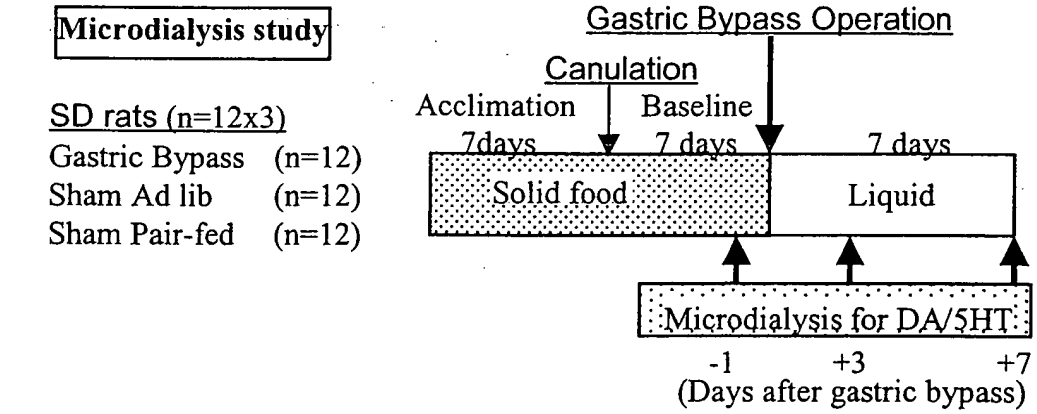
Experiment #4 (Agonist study): To verify our hypothesis that the decrease in ghrelin (afferent feeding stimulatory signal) induces the early satiety and the decreases in food intake and body weight, we plan to examine the effects of peripheral administration of ghrelin (an agonist for growth hormone secretagogue receptor) on food intake, body weight and carcass composition in obese Zucker rats.

Figure 16: Experiment #4 for specific aim #1



Obese Zucker (n=72) and lean rats (n=72) will be acclimated for two weeks and studied in ACREM. After one week of baseline data, rats will be operated for gastric bypass or sham operation under anesthesia. Zucker rats will be fed liquid diet for one week after the operation and then, changed to solid food while remaining in the ACREM throughout. Gastric bypass or sham operated rats will be intraperitoneally injected with ghrelin dissolved in saline (pH 7.4) at one or three weeks after the operation, and body weight, meal size and meal number will be measure using ACREM. Carcass analysis will be determined at the end of the experiment.

Figure 17: Experiment #5 for specific aim # 3



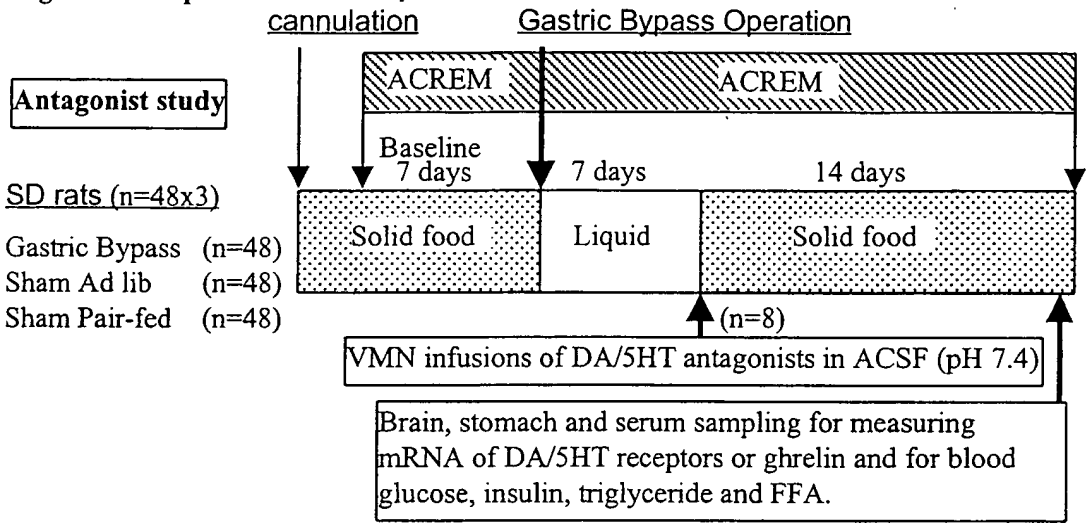
Experiment #5 (Microdialysis Study): Our previous reports demonstrated that DA concentrations in ventromedial hypothalamic nucleus (VMN) are associated with meal number using microdialysis in SD rats, the meal number might be increased by gastric bypass operation. Thus, we need to investigate the effects of gastric bypass operation on changes in DA and/or 5HT concentrations in the VMN via the use of in vivo microdialysis technique.

As shown in schema, after two week acclimation, SD rats (n=36) will be implanted with the VMN guide cannulas for microdialysis **(see Methods)**. One week after cannulation, first microdialysis will be performed for 24 hrs starting at 1 pm, after microdialysis probe insertion at 9 am to allow for sufficient stabilization period.

Each 20-min dialysate will be immediately analyzed for DA and 5HT concentrations by HPLC, in order to measure the baseline levels of DA and 5HT in SD rats **(see Methods)**. Then, rats will be undergo operation for gastric bypass or sham according to the schema and fed liquid diet for one week. A second and third microdialysis will similarly be performed 3 and 7 days after operation and DA and/or 5HT levels will be measured.

Experimental # 6 (Antagonist study): The purpose of this study is to examine the effect of gastric bypass operation on the hypothalamic aminergic system using four specific antagonist, two for DA (D1 and D2) and two for 5HT (1B, and 2C) receptors. This will necessitate a significant number of rats, but the information derived from these crucial studies is invaluable in ultimately developing effective therapeutic counter measures to obesity.

Figure 18: Experiment #6 for specific aim #3

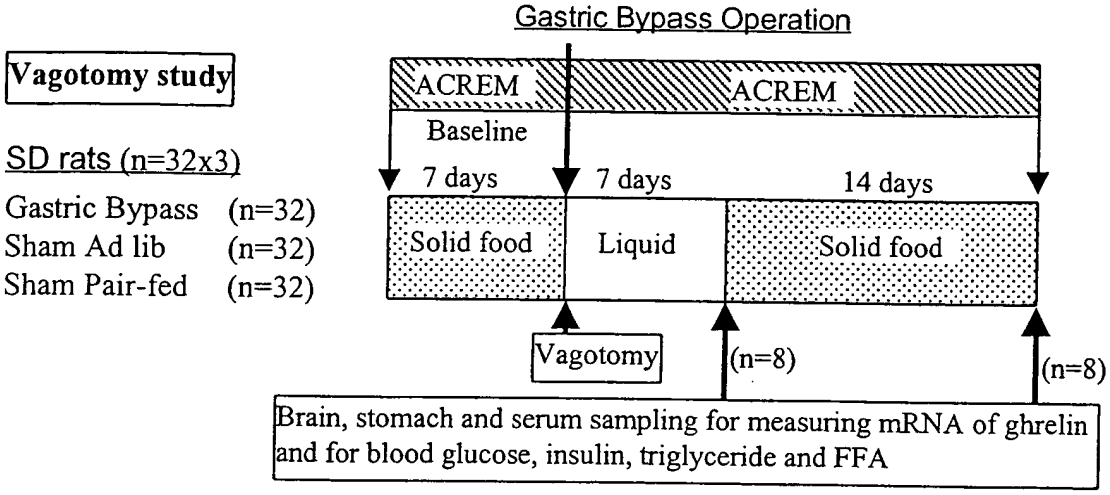


As shown in the schema, SD rats (n=384) will be implanted with VMN guide cannulas connected to osmotic minipump placed subcutaneously and studied in ACREM to measure meal size and number (12 rats in each experiment; receiving either the antagonist or normal saline as control). After two weeks of acclimation, and after one week of baseline data, rats will be operated on for either gastric bypass or sham, fed liquid diet for one week while being studied in the ACREM. Receptor antagonists (one antagonists per 12 gastric bypass, 12 sham ad lib and 12 sham pair-fed rats): for D1 DA receptor –SCH (Sigma Chemicals, St Louis, MO), D2 DA receptor- Sulpiride (Sigma, St Louis, MO), 5-HT1B receptor – MM 77 (Tocris, Ballwin, MO) or 5HT2C receptor – Mianserin (Sigma, St Louis, MO), or vehicle (n=12) will be chronically delivered (14 days, 4μg/0.5μl per hour) into the VMN for two weeks. Four separate experiments will be performed to evaluate the effects of specific antagonist for dopamine D1, D2, serotonin 5-HT1A or 5-HT2C receptors on meal size, meal number, daily food intake and body weight gain in obese and lean Zucker rats. Rats will be killed by decapitation under anesthesia three weeks after the gastric bypass operation. Brain and stomach will be immediately removed, hypothalamus and fundus will be dissected, and blood sample will simultaneously be taken **(see Methods)**. The ghrelin mRNA will be extracted from the homogenates of hypothalamus and stomach and RT-PCR will be performed. Gels will be scanned and analyzed by comparing with corresponding expression of β-actin in the same brain and stomach samples using Sigma Gel computer program. Blood glucose, insulin, triglyceride and FFA will be measured using enzymatic colorimetric assay, and carcass analysis will be done in a select number of rats.

Experiment #7 (Vagotomy study): The purpose of this experiment is to determine the role which the vagus plays in sending gastric ghrelin signals to the hypothalamus, as it relates to reduced gastric capacity and a degree of malabsorption. For this reason, as the schema shows, rat will need to be sacrificed in the short term after operation and in the long term, thus requiring the necessary number of rats.

96 Sprague Dawley rats will be studied in ACREM to measure meal size and meal number after one week of acclimation. As depicted in scheme, after a week of baseline data, rats will be operated for gastric bypass or sham and vagotomy or no vagotomy.

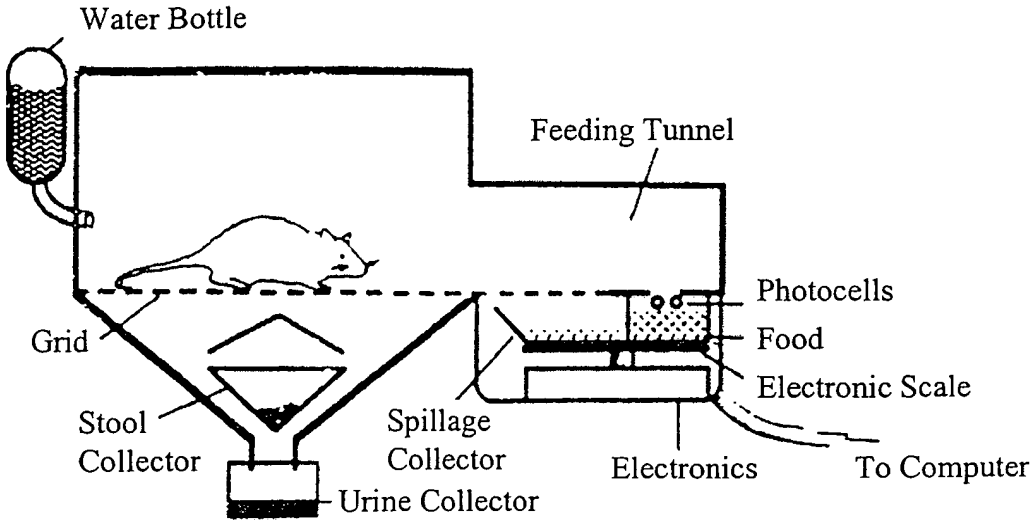
Figure 19: Experiment #7 for specific aim #2



They will be killed by decapitation under anesthesia one or three weeks after the operation (n=16 rats each per experimental group). Brain and stomach will be immediately removed, hypothalamus and fundus will be dissected, and blood sample will simultaneously be taken. Ghrelin mRNA will be extracted from tissue homogenates and RT-PCR will be performed **(See Methods).** Gels will be scanned and analyzed by comparing with corresponding expression of β-actin in the same brain and stomach samples using Sigma Gel computer program. Blood glucose, insulin, triglyceride and FFA will be measured using enzymatic colorimetric assay and select number of rats will undergo carcass analysis **(See Methods).**

METHODS

Automated Computerized Rat Eater Meter (ACREM): We designed and built ACREM to enable us to measure each discrete access to food and to distinguish between an access which results in food consumption and one which does not (65). Through computerized calculations, the ACREM permits continuous recording of meal number, meal size and food intake, for either 24 hours or for each light/dark cycle in the spontaneous free feeding rat without the need for pretraining or reflex conditioning. It is equally applicable to normal and to pathophysiological rat models. The ACREM is based on a commercially available metabolic cage in which the standard feeding cup was replaced with a unit containing an electronic strain-gauge balance for weighing the food. A combination food dish and spillage-collector bowl was placed on the balance. Photoelectric sensors were placed at the end of the tunnel just above the food dish to monitor access to chow. The entire unit was housed in a 6x10x16 cm sealed aluminum box, which slid under the feeding tunnel on the rails provided for the commercially supplied food cup. A real-time remote computerized data collection device was used to integrate the information on feeding characteristics as detected from the photoelectric cell and the electronic scale with real-time.



Schematic illustration of Automated Computerized Rat Eater Meter consisting of a commercially available metabolic cage with a feeding tunnel modified by an electronic food scale and photo cells.

Animals: Adult male Sprague Dawley and male obese (*fa/fa*) and lean (Fa/Fa) Zucker rats 2.5-3 months old were purchased from Harlan (Indianapolis, IN). Rats were housed in holding wire mesh cages for 1 week after purchase to acclimate them to the constant study environmental conditions: 12-h light cycle (06.00-18.00 h) 26±1°C room temperature, 45% humidity.

Diets: Solid food: Standard rat chow (Diet 5008; Ralston Purina, St. Louis, MO). Liquid food: Boost, Nutritional energy drink. Mead Johnson, Evensville, IN. Providing one cal/ ml. In 240ml there are 41g carbohydrate, 49 fat and 10g protein. Tap water is available ad libitum.

Anesthesia: Microdialysis guide cannula placement are performed under general anesthesia obtained with a mixture of Ketamine HCl 150mg (1.5ml)+ Xylazine 30mg (1.5ml) + Acepromazine 5mg (0.5ml), administered subcutaneously at a dose of 0.5-0.7 ml/kg. Anesthesia overdose is used to sacrifice the rats.

Specific Receptor Antagonists: Dose 4μg/0.15μl/h: for DA D1 receptor-SCH 23390 (Sigma Chemicals, St. Louis, MO), for DA D2 receptor-Sulpiride (Sigma, St. Louis, MO), for5-HT1B receptor – Isamoltane (Tocris, Ballwin, MO).

Ghrelin: This peptide will be provided by Dr. Inui (see letter of collaboration). In addition he will provide anti-NPY antibodies for histological studies.

Carcass analysis to assess Body Composition for Fat Mass: This technique has been extensively used in our laboratory and data thereof have been published. The fat content of a rat is determined using the method of Hartsook and Hershberger (66). Pre-weighed rats are autoclaved for 15 minutes. After that, each rat is homogenized in twice its total weight of distilled water using a blender and a homogenizer. An aliquot of homogenate is then put in pre-weighed test tube, and a methanol:chloroform (2:1) solution is added and vortexed and left at room temperature for an hour. After that, chloroform and KCl are added and vortexed and stayed in ice for 15 minutes. The test tube is centrifuged. The chloroform layer, the bottom of three layers, is poured into a pre-weighed pan, and is placed in a fume hood until the chloroform evaporates. Approximately 12 hours later, the pan is re-weighed to determine the amount of fat contained within the pan and total fat weight can be calculated.

Blood Glucose, Triglyceride, Free Fatty Acid (FFA) and Insulin: Rats will be killed by decapitation under isoflurane (Forane, Baxter, Chicago,IL) anesthesia within 2 min. Blood samples will be taken into EDTA-rinsed tubes and centrifuged 3,000 rpm for 10 min at 4°C. The plasma samples are stored at –80°C before analysis. Glucose and triglyceride levels will be measured by enzymatic colorimetric kit (Sigma, St. Louis, MO). FFA levels also will be quantified by enzymatic spectrophotometric kit (NEFA Wako,Richmond, VA). Immunoreactive plasma insulin will be measured using enzyme-immunoassay (Mercodia Rat Insulin ELISA, ALPCO, Windham, NH).

Dopamine and Serotonin Collection and Measurement: Microdialysis technique allows us to collect serotonin and dopamine sample continuously from the VMN or the LHA in a freely moving rat.The CMA/10 microdialysis probe is purchased from BAS (West Lafayette, IN). The microdialysis probe is 1 mm long, 400 μm ID, 520 μm OD and 20,000 Dalton molecular weight cut-off. According to our in-vitro calibration test, a relative recovery rate is about 10-12% for serotonin and dopamine at a flow rate of 1 μl/min. The sample is collected from 20 min dialysates. The perfusion is performed with a Ringer type solution containing 147 mM Na^+, 2.3 mM Ca^{++}, 4 mM K+ and 155.6 mM Cl^-. Allowing a 60-min stabilization period, three 20-min dialysates are collected. Flow rate, 1μl/min, allows collection of 20 μl samples every 20 min into the microvial. Serotonin and dopamine are detected using a reverse-phase liquid chromatography with ESA Model 5014 high sensitivity analytical cell and ESA Hypersil ODS column (3 μm; 150 x 3 mm). Mobile phase consists of 75 mM $NaH_2PO_4 \cdot H_2O$, 1.4 mM OSA, 10 μM EDTA, and 10% acetonitrile. Buffer pH is adjusted to 3.5 with H_3PO_4.

Verification of Cannula Placement: At the end of certain microdialysis experiments rats are killed with an anesthetic overdose. Then, each rat is perfused via the ascending aorta with saline solution followed by 10% formaldehyde solution to fix the brain. The harvested brain is further fixed in 40% formaldehyde overnight, and then serial coronal sections are cut through the hypothalamic region. The tissues are stained with cresol violet, and examined by light microscopy to confirm appropriate anatomical placement of microdialysis catheters.
Microdialysis Guide Implantation: The rat is anesthetized with a mixture of Ketamine, Xylazine and Acepromazine (150:30:5 mg/ml at 0.7 ml/kg body weight given intramuscularly) and is placed in a stereotaxic frame (Kopf Instruments) with the horizontal zero plane tangential to the upper incisor bar and 3.3 mm below the interaural line. A VMN cannula (CMA/12 CMA/Microdialysis, Acton, MA) will be implanted using the coordinates: VMN: 2.0 mm posterior to the bregma; media-lateral, 0.7 mm from the middle line; and 8.0 mm ventral from the surface of the dura mater respectively. The cannula guide is fixed to the skull with acrylic dental cement.

Microdialysis Procedure: A CMA/10 microdialysis probe is used (CMA/Microdialysis, Acton, MA). The microdialysis membrane is 1 mm long, 400 μm ID, 520 μm OD and 20,000 Dalton molecular weight cut-off. According to our *in vitro* calibration test, the relative recovery rate for dopamine/serotonin is about 10-12% at a flow rate of 1 μl per min. A Ringer type solution containing 147.0 mM Na^+, 2.4 mM Ca^{2+}, 4.0 mM K^+ and 155.8 mM Cl^- is used for perfusing by a CMA/100 microinjection pump (CMA/Microdialysis, Acton, MA). The flow rate of 1 μl/min will allow the collection of a 20 μl sample into microvials every 20 min.

Experimental Procedure During Microdialysis: On the experimental day, the rat is placed into a bowl-like cage (CMA/120 Awake Animal System, BAS, West Lafayette, IN) and a microdialysis probe is inserted into the guide cannula, extending 1 mm beyond the cannula into the nucleus under study. After a 4 h stabilization period, 20-min dialysates are collected for 24 hours and immediately analyzed. Dopamine and 5-HT are detected using a reverse-phase liquid chromatography with ESA Model 5014A high sensitivity analytical cell and ESA MD-150 column (15 cm x 3.0 mm ID). The mobile phase consists of 75 mM $NaH_2PO_4 \cdot H_2O$, 1.8 mM OSA, 10 μM EDTA, and 11% acetonitrile. The pH of the buffer is adjusted to 3.0 with H_3PO_4. The levels of dopamine and 5-HT are expressed in pg per 10 μl dialysate.

In Situ Hybridization: To examine the expression of ghrelin, NPY and AGRP mRNA in hypothalamic nuclei after gastric bypass operation, in situ hybridization will be performed.

i) Tissue Preparation: Rats will be euthanized via a lethal dose of a mixture of Ketamine, Xylazine and Acepromazine and perfused via the ascending aorta with 50 ml (at 37°C) of 0.9% NaCl solution followed by 250 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.5. The brains will be rapidly dissected, immersion-fixed in the fixative for one night, rinsed in 0.05 M TRIS-buffered saline (TBS), pH 7.4, and by 10% and 20% sucrose in TBS for at least 24 h. The brains will be cut serially into 20 μm thick frontal sections on a cryostat and rinsed in 0.1 M PBS.

ii) Oligonucleotide Radiolabelling: Oligonucleotide antisense probes (and sense probes as specificity controls) complementary to ghrelin, NPY or AGRP mRNA (see Table) will be radiolabelled using Terminal deoxynucleotidyl Transferase (TdT) (Boehringer-Mannheim, Indianapolis, IN). Two picomoles of each oligonucleotide will be incubated with 20 μCi ^{35}S deoxyadenosine triphosphate (Amersham Corp., Arlington Heights, VA. 10 mCi/ml) and 25 units TdT in 10 μl of buffer (100 mM potassium cacodylate, 2 mM cobalt chloride, 0.2 mM dithiotreitol). The reaction is stopped in 0.2 M EDTA, 5 μg/μl yeast tRNA (Sigma, St. Louis, MO) solution after incubation for 45 minutes at 37°C and the probe will precipitated with 4M chloride lithium and cold ethanol.

Immunohistochemistry.
To visualize dopamine D1, D2 receptors; serotonin 5-HT1B, 5-HT2C receptors in the hypothalamic nuclei after gastric bypass operation we will use Peroxidase-antiperoxidase (PAP) or Avidin-biotin(ABC) method.

i) Tissue Preparation: Rats will be killed via a lethal dose of a mixture of Ketamine, Xylazine and Acepromazine and perfused via the ascending aorta with 150 ml of 0.9% NaCl solution followed by 250 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3-7.4 (PAF). The brains will be dissected from the scull. The part containing hypothalamus will be immersion-fixed in the PAF for 2-4 hours, rinsed in 0.02 M phosphate-buffered saline (PBS), pH 7.3-7.4, and soaked in 30% sucrose in PBS for at least 24 h. The brains will be freezed in dry ice and stored at -70°C before using. Hypothalamus will be sectioned serially into 10-20 μm thick Immunohistochemistry:
The hypothalamic sections will be processed for immunohistochemistry of:
Dopamine receptors DA_{1-2}, will be visualized with the antiserum raised in goat (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) followed by goat ABC kit (Vector) and developing with DAB-kit (Vector, Burlingame, CA).

Serotonin receptors 1A and 1B will be visualized with the antiserum raised in guinea pig (Chemicon Int.Inc., Temecula, CA) and anti-guinea pig secondary antibodies and guinea pig PAP complex (Jackson Imm.Res. Labs. West Grove, PA) using DAB as a chromogen.

Main steps PAP/ABC immunohistochemical methods consists of several incubations of the slides with washing steps in PBS between them: preincubation with 0.3% H_2O_2 for 30 min; rinsing in PBS (3 x 10 min), incubation in 2% bovine serum albumine (BSA) for 30 min; primary antibodies at dilutions 1:400 – 1:2000 for12-48 h in the refrigerator; rinsing; secondary antibodies 1-2 h at room temperature; rinsing; PAP or ABC complex 2 h at room temperature; rinsing; visualization of the reaction product with DAB or 4-chloro-1-naphthol; washing; dehydration and mounting the sections into the Fisher Permount Mounting Medium.

iii)Immunofluorescent doublelabeling:
Incubation with 2% BSA 30 min than in mixture of 2 primary antibodies for 12-48 h in the refrigerator, rinsing , incubation for 2h with secondary antibody labeled FITC (diluted 1:300; Anti-rabbit IgG FITC CONJUGATE developed in goat or Anti guinea-pig IgG FITC CONJUGATE developed in rabbit , SIGMA), washing and next 2 h secondary antibody labeled TRITC (dilution I:300, Anti-rabbit IgG TRITC CONJUGATE developed in goat, SIGMA). After last washing sections embedded in VECTASHIELD Mounting Medium (Vector Laboratories,

Burlingame, CA) The result of reaction will be analyzed with the fluorescent microscope with filter sets for fluoresceine and rhodamine or in confocal microscope.

Table I: Probe

Gene	Probe	Position	Reference
Ghrelin	5'-GCT TTC TGG TGC TCT GGG CTC AAG AAG CTG GAA CCT GCC ATG GCC-3'	90-134	(4)
	5'-TGC TGG GAG TTG CAG AGG AGG CAG AAG CTG GAT GTG AGT TCT TGC-3'	421-465	(4)
NPY	5'-GCT CTG CGA CAC TAC ATC AAT CTC ATC ACC AGA CAG AGA TAT-3'	222-263	Gene Bank (M20373)
	5'-GGA GTA GTA TCT GGC CAT GTC CTC TGC TGG CGC GTC-3'	201-166	(67)
AGRP	5'-CTG CTG CTG TCT TCT TCA GAC TTA GAC CTG AGA ACT CTG GGA ATA GGG-3'	1-48	(68)

RT-PCR

Rats will be killed by the decapitation; brain and stomach are rapidly removed and hypothalamus tissue are dissected and homogenized in 250 μl RNA pure™ reagent (Gen Hunter Corporation, Nashville, TN), using a Sonic Dismembrator 550 (Fisher Scientific, Houston, TX.). Total cellular RNA is isolated by RNA pure™ reagent (Gen Hunter Corporation, Nashville, TN), RNA pellets are rinsed with 70% ethanol, resuspended in 10 μL of DEPC-treated water. RNA concentration quantitated by absorbance at 260 nm. 2.0 μg of total RNA from each sample are reverse transcribed to cDNA by priming with oligo dT poly-nucleotide using SuperScript II (Life Technologies, Gaithersburg, MD). 4 μl of each RT product is amplified by PCR using primers chosen according to the published rat coding sequences for ghrelin, NPY or AGRP and for dopaminergic D1, D2, serotonergic 5HT1B or 5HT2C, see Table below. The quantitation is based on internal RNA standard-expression of housekeeping β-actin gene (69), see Table below. To confirm the purity of RNA extraction and exclude the possibility of DNA contamination, we propose to use RNA as a template in PCR reaction without prior reverse transcription. In order to examine whether contamination of reagents occurred in the present experiments, distilled water is simultaneously subjected to RT-PCR (RT-PCR blank).

Table II. PCR Primers

Gene	Primer	Product size bp	Reference
Ghrelin	5'-GGACATGGCCATGGCAGGTT-3' 5'-TTGTTAGCTGGCGCCTCTTT-3'	296	GenBank AB 02944
NPY	5'-TAGGTAACAAACGAATGGGG-3' 5'-AGGATGAGATGAGATGTGGG-3'	351	(70)
AGRP	5'-AGGGCATCAGAAGGCCTGACCAGG-3' 5'-CTTGAAGAAGCGGCAGTAGCACGT-3'	590	(71)
DA D1 receptor	5'-CAGTCCATGCCAAGAATTGCC-3' 5'-AATCGATGCAGAATGGCTGGG-3'	225	(72)
DA D2 receptor	5'-GCAGCTGAGCTTTCAGAGCC-3' 5'-TCTGCGGCTCATCGTCTTAAG-3'	404 (long) 317(short)	(73,74)
5HT1B receptor	5'-GTTGACTTGTCAATGGCAT-3'	200	(75)
5HT2C receptor	5'-GCTTCAGTTCACATTCCAGA-3' 5'-ATTACTTCTTAATGTCCCTAGCCATTGCTGA-'3 5'-TATTTGTGCCCCGTCTGG-3'	484	(76,77)
β-actin	5'-ATGGATGACGATATCGCTGCG-3' 5'-CTCCATATCGTCCCAGTTGGT-3'	249	(72)

The PCR will be performed in a final volume of 50 µl containing 1,5 mM $MgCl_2$, 350 µM dNTP, 10 pmol of each primer, and 2.5 units of *Taq* DNA polymerase (Life Technologies, Gaithersburg, MD) using PCR thermal cycler (Perkin Elmer 9700) as follows: denaturation at 94°C for 1 min, annealing for 1 min and elongation at 72°C for 1 min. The PCR products are electrophoresed on 2 % agarose gel in 0.5x Tris-Borate-EDTA (TBE) buffer and evaluated via ethidium bromide staining (0.5 µg/ml).

Photo image will be scanned using the transilluminator and the gel imaging system (Genelink, Spectronics corporation) and the level of expression will be quantified by Gel analyzing software (Sigma Gel). The ratios of ghrelin, NPY or AGRP and for dopaminergic D1, D2, serotonergic 5HT1B or 5HT2C receptors to β–actin are calculated and the effects of gastric bypass operation on their expressions are examined.

STATISTICAL ANALYSIS AND DATA HANDLING

This proposal contains studies to understand the process of weight loss associated with the gastric bypass operation. Food intake behavior, body weight as well as physical and chemical changes in stomach and in brain samples respectively, influenced by the treatment factor based on measurements of rats under experimental conditions will be collected and analyzed. Three feeding indices (food intake, meal size, meal number) and body weight are continuously measured for a prolonged time of several weeks. Body weight and the average eating behavior measurements will be analyzed by the time-series models suggested by Box, Jenkins and Reinsel (78). Graphical methods as well as various descriptive statistics would be applied to describe the empirical patterns of these variables. Time series and regression models are developed to represent the averaged eating behavior and its association with the body weight in the control group and the study group respectively. The difference (or the effect of the treatment) between the control group and study group can be estimated in terms of parameters of the estimated time series regression models. Measurements of ghrelin in the stomach and changes of dopamine and serotonin concentrations, NPY and AGRP are measured on selected days, as indicated in the experimental protocols, as outlined in **SECTION D. RESEARCH DESIGN AND METHODS**, above.

Descriptive analysis will be performed. Analysis of variance (ANOVA) procedures then can be applied to test the significance of the treatment in the possible physical and chemical changes., and correlation between them performed, to ascertain the functional relevance of their biological activity.

EXPECTED OUTCOME: Every method posed in this proposed programmatic investigation has already been accomplished successfully in this laboratory. Therefore, we anticipate no significant experimental problems or delays related to the methods. We believe that the anticipated results will support our hypothesis. We expect that they will also raise further points of interest. However, we recognize that the set of experiments set forth in this programmatic investigation will not be conclusive when considered separately. Also, we recognize that the proposed research, when completed, will not have solved the multifactorial mechanisms leading to morbid obesity, but it will have shed some light on the role played by i) relevant anatomical areas, ii) the different neurotransmitters, and iii) ghrelin, as affected by gastric bypass procedure and progressive weight loss.

The previously reported studies and the investigations outlined in the **Preliminary Studies and Background Section** indicate that: i) our laboratory skills and analytical methods are suitable for this proposed effort; and ii) the proposed results, which are based on the stated hypothesis, are likely to be forthcoming when our currently established methods are applied to the experimental design. Based on our initial preliminary studies we anticipate that the results of the experiments outlined in this proposal will characterize the involvement of hypothalamic mechanisms in morbid obesity as determined by specific changes in ghrelin, dopamine and serotonin, their receptors density and distribution.

CONTINGENCIES: Every method posed in this proposed programmatic investigation has already been accomplished successfully in this laboratory. Therefore, we anticipate no significant experimental problems or delays related to the methods. Material–related contingencies will be solved as follows: unexpected death of animals during anesthetic or surgical procedures may potentially bias the statistical relevance of the results. To solve this problem, we are including in each experiment an adequate number of animals to obtain data with statistical power, plus 10% attrition. A critical step in the successful completion of the programmatic investigation here proposed is the statistical analysis of the results obtained. This task will be accomplished by our dedicated statistician, Professor Chung Chen, using the above-mentioned statistical analysis. We believe that the anticipated results will support our hypothesis. We expect that they will also raise further points of interest. However, we recognize that the set of experiments set forth in this programmatic investigation will not be conclusive when considered separately.

SECTION E. HUMAN SUBJECTS – Not applicable.

SECTION F. VERTEBRATE ANIMALS

A total of 384 adult male Sprague Dawley rats, 216 obese Zucker and 216 lean Zucker rats will be studied. In our proposal, "Gastric Bypass in Obesity: Ghrelin-Related Weight Loss" our long-term objectives continue to be the elucidation of mechanisms underlying medically significant food intake abnormality, through studies of relevant animal models, particularly that of morbid obesity as compared to normal controls. As the title and the objectives of the study suggest, the thrust of these investigations involves changes in brain biochemistry as they relate to gastric stapling procedure and the induction of chronic malabsorption to produce chronic weight loss. Thus, an intact animal model needs to be used; the needed specimens cannot be obtained from humans. Numerous previous studies in this area have traditionally used the rat model because: a) it is still relatively inexpensive; and b) a considerable body of knowledge exists regarding rat metabolism and food intake behavior. For a complete description of proposed use of rats, see **Experimental Design** section. It should

be noted the University Hospital has extensive animal facilities (see **Resources and Environment**), a Committee for the Humane Use of Animals, and full-time veterinary services which ensure that discomfort and injury to the rat is limited to that which is unavoidable in the conduct of scientifically valuable research, and that anesthetic drugs are used where indicated and appropriate to minimize discomfort and pain to the rat. Rats are operated under general anesthesia using traditional aseptic antiseptic surgical techniques. When rats are sacrificed at the end of the study, this is done by the anesthetic overdose method or by decapitation under general anesthesia.

SECTION G. PROPOSED TIMETABLE

Experiment No.	1	2	3	4	5	6	7	
	SD	Zucker	Histology	Agonist	Micro-	Antagonist	Vagotomy	Total
					dialysis			
Acclimation (3rats/cage)	1	1		1	1	1	1	6
Individual cage	4	4	4	4	2	4	4	26
Clean up	1	1	1	1	1	1	1	7
Subtotal (wks)	6	6	5	6	4	6	6	39
Experimental times	2.25	4.5	4.5	4.5		4.5	3	23.25
Total w/out analysis (wks)	**13.5**	**27**	**22.5**	**27**	**40**	**27**	**18**	**175**
Analysis time (weeks)	8.6	39.9	96.0	0.0	0.0	38.9	11.5	193.9
Total (wks)	**22.1**	**66.9**	**118.5**	**27.0**	**40.0**	**65.9**	**29.5**	**368.9**
					Total-Exp#3 and #6(weeks):			**210.4**
								4.38 year

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